~~PRODUCTS AND PROCESSES FOR MODULATING PEPTIDE-PEPTIDE BINDING DOMAIN INTERACTIONS~~

COMPUTER COMPRISING ATOMIC COORDINATES OF A PLK-1 POLO-BOX DOMAIN AND USES THEREOF

Cross-Reference to Related Applications

This application claims the benefit of U.S. provisional patent applications 60/426,132, filed November 14, 2002, 60/485,641, filed July 8, 2003, and 60/487,899, filed July 17, 2003.

Statement as to Federally Sponsored Research

The present research was supported by a grant from the National Institutes of Health-National Institute of General Medical Sciences (NIH-NIGMS; grant number GM52981). The U.S. government has certain rights to this invention.

Background of the Invention

The invention relates to compounds (*e.g.*, peptidomimetics and non-peptides) that inhibit a cellular proliferative disorder and methods of treating such disorders. The invention also provides three-dimensional structures of a Polo-like kinase and methods for designing or selecting small molecule inhibitors using these structures. Desirably, these compounds have certain structural, physical, and spatial characteristics that enable the compounds to interact with specific amino acid residues.

Cyclin-dependent kinases (Cdks) have long been considered the master regulators of the cell-cycle, but an increasing number of diverse protein kinases are now emerging as critical components of cell-cycle progression. Among these are members of the Polo-like kinase family (Plks) that play key roles during all

stages of mitosis and in the cell cycle checkpoint response to genotoxic stress. Many protein kinases involved in cell-cycle control function, in part, by generating phosphoserine/threonine-containing sequence motifs in their substrates that are subsequently recognized by phosphoserine/threonine-binding proteins. These include the WW and proline isomerase domain of Pin1 that regulates mitotic progression, 14-3-3 proteins that control the G2/M transition in response to DNA damage, and the WD40 repeat of Cdc4p which regulates S-phase entry.

In several instances, a phosphopeptide-binding domain and a kinase domain are combined within a single molecule, best exemplified by the SH2 domain-containing Src kinases and the Rad53p/Chk2-family of FHA domain-containing kinases. In these proteins the phosphopeptide-binding domain targets the kinase to pre-phosphorylated (primed) sites, mediates processive phosphorylation at multiple sites within a single substrate, or facilitates kinase activation. Polo-like kinases are distinguished by the presence of a conserved Ser/Thr kinase domain and a non-catalytic C-terminal region composed of two homologous ~70-80 residue segments termed Polo-boxes.

Humans, mice and frogs each have three Plk homologues denoted Plk1, Plk2/Snk, and Plk3/Fnk/Prk, while budding yeast, fission yeast, and flies contain only a single Plk family member denoted Cdc5p, Plo1, and Polo, respectively. In addition, humans and mice have a serine/threonine kinase, Sak, that is an extremely divergent member of the Plk family, containing only a single Polo-box and lacking a canonical PBD.

The most extensively studied Polo-like kinases, Plk1 and Cdc5p, have been implicated in numerous mitotic processes including activation of Cdc25C and Cdc2-cyclinB at the G2-M transition, centrosome maturation and spindle assembly, cohesin release/cleavage during sister chromatid separation, anaphase promoting complex (APC) activation during mitotic exit, and septin regulation during cytokinesis. In contrast human Plk2 and Plk3 appear to serve different functions. Plk2 shows peak expression and activity in early G1, while Plk3 is

activated by several stress response pathways, including DNA damage and spindle disruption. In fact, Plk3 plays some roles that may directly antagonize Plk1 function. For example, DNA damage directly inhibits Plk1, but activates Plk3 in an Ataxia-Telangiectasia-Mutated (ATM)-dependent manner. Consistent with these results, Plk1 overexpression causes oncogenic transformation in NIH 3T3 cells, while overexpression of Plk3 induces apoptosis.

Summary of the Invention

We have developed a proteomic approach for identifying targets downstream of kinases in signaling pathways. Our strategy involves using an immobilized library of partially degenerate phosphopeptides, biased toward a kinase phosphorylation motif, to isolate interacting effector proteins targeted by substrates of that kinase. Utilizing this approach for cyclin-dependent kinases, we discovered that the carboxy-terminal region of the cell cycle regulating kinase, Plk-1, encodes a phosphopeptide recognition domain that consists of the non-kinase region of this protein (amino acids 326-603). This phosphopeptide recognition domain, termed the Polo-box domain (PBD), binds phosphoserine and phosphothreonine residues in a sequence-specific context. Specifically, this PBD recognizes and binds to the core phosphopeptide sequence serine-phosphoserine or serine-phosphothreonine.

We performed oriented peptide library screening on the PBDs from all three human Plk homologues, as well as on the Plk1 orthologues Plx1 from *Xenopus* and Cdc5p from budding yeast. Despite differences in cellular function, we found that all PBDs show strong conserved selection for the core sequence S-[pSer/pThr]-P/X.

To determine the structural basis of PBD activity, the crystal structure of the human Plk1 PBD in complex with its optimal phosphothreonine-containing peptide was determined. We identified a mode of phosphopeptide binding that is

unique among structurally characterized phosphodependent binding protein/modules and that is crucial for PBD targeting to substrates both *in vitro* and *in vivo*. The architecture of the Plk1 PBD differs significantly from that recently observed for homodimers of the single Polo-box from murine Sak, which lacks a formal PBD (Leung et al., Nat. Struct. Biol. 9:719-724, 2002). The Plk1 PBD represents a new protein fold. Site-directed mutagenesis based on the structural identification of critical phosphothreonine-binding residues has enabled us to demonstrate that phosphodependent substrate recognition by the PBD is necessary for proper mitotic progression. Furthermore, binding of the optimal Plk1 phosphopeptide to the PBD in full-length Plk1 enhances the *in vitro* activity of the kinase domain, leading to a model for Plk regulation in which intramolecular inhibition of the kinase by the PBD is relieved by PBD-ligand binding. We conclude that phosphoserine/threonine-dependent binding is a general feature of PBD activity across the Plk family and critically important for the function of this domain in Polo-like kinase targeting and regulation. These studies have identified sites that may be targeted in designing therapeutics useful in treating diseases or disorders characterized by inappropriate cell cycle regulation or inappropriate cell death.

We applied the same proteomic approach to identify phosphopeptide-binding modules mediating signal transduction events in the DNA damage response pathway. Using a library of partially degenerate phosphopeptides biased to resemble the phosphorylation motif of the phosphoinositide-like kinases ATM and ATR, we identified tandem BRCT domains in PTIP and BRCA1 as phosphoserine (pSer)- or phosphothreonone (pThr)-specific binding modules that recognize a subset of ATM (ataxia telangiectasia–mutated) and ATR (ataxia telangiectasia– and RAD3-related) -phosphorylated substrates following γ-irradiation. PTIP tandem BRCT domains are responsible for phosphorylation-dependent protein localization into 53BP1-and phospho-H2AX (_-H2AX)–containing nuclear foci, a marker of DNA damage.These findings provide a new

molecular rationale for BRCT domain function in the signaling response to DNA damage and may help to explain why the BRCA1 BRCT domain mutation Met1775 3 Arg, which fails to bind phosphopeptides, predisposes women to breast and ovarian cancer..

In one aspect, the invention generally features computer containing a processor in communication with a memory; the memory having stored therein (i) at least one atomic coordinate, or surrogates thereof, from Table 5 for each of the following residues: His-538, Lys-540, Trp-414, or Leu-491 of a Polo-box domain or atomic coordinates that have a root mean square deviation of the coordinates of less than 3 Å; and (ii) a program for generating a three-dimensional model of the coordinates. In one embodiment, the coordinate is for a heteroatom. In another embodiment, the coordinate is for a side-chain atom. In another embodiment, the coordinate is for a side-chain and a heteroatom.

In another aspect, the invention generally features a computer containing a processor in electrical communication with a memory; the memory having stored therein (i) atomic coordinates, or surrogates thereof, as shown in Table 5 for atoms of residues His-538, Lys-540, Trp-414, or Leu-491 of a Plk1 Polo-box domain or atomic coordinates that have a root mean square deviation from the cooridinates of the residues of less than 1, 2, 3, 4, or 5 Å; and (ii) a program for displaying a three-dimensional model of the Polo-box domain.

In another aspect, the invention provides a computer containing a processor in communication with a memory; the memory having stored therein (i) x-ray diffraction data for at least one of the non-hydrogen atoms of residues His-538, Lys-540, Trp-414, or Leu-491 of a Polo-box domain or x-ray diffraction data for amino acids that have a root mean square deviation from the backbone atoms of the residues of less than 1, 2, 3, 4, or 5 Å; and (ii) a program for generating a three-dimensional model of the Polo-box domain.

In another aspect, the invention provides a computer containing a processor in communication with a memory; the memory having stored therein a

pharmacophore model of a phosphopeptide that binds a Polo-box domain and a program for displaying the model, the model containing at least one of the following: a phosphate group on threonine that participates in at least 1 hydrogen-bonding interaction; and a serine at the pThr-1 position, where the Ser-1 side chain is directed towards the Plk1 surface. In one embodiment, the serine engages in at least two of the following (i) a hydrogen bonding interaction with Trp-414 main-chain atoms of PBD; (ii) a hydrogen bonding interaction with Leu-491 main-chain carbonyl of PBD; and (iii) a van der Waals interaction with Cδ1 from the Trp-414 indole side chain of PBD. In one embodiment, the model further comprises a Proline at the pThr+1 position, where the proline introduces a kink that allows a pThr+2 main chain amino group to contact PBD.

In another aspect, the invention provides a method of selecting or designing a candidate ligand for a Polo-box domain, the method involves the steps of: (a) generating a three-dimensional structure of a Polo-box domain having at least one atomic coordinate, or surrogate thereof, from Table 5 for each of the following residues: His-538, Lys-540, Trp-414, or Leu-491or atomic coordinates that have a root mean square deviation from the coordinates of less than 1, 2, 3, 4, or 5 Å; and (b) selecting or designing a candidate ligand having sufficient surface complementary to the structure to bind a Polo-box domain in an aqueous solution. In another aspect, the invention provides a method for manufacturing a Polo-box domain ligand, the method involves the steps of: (a) obtaining the atomic coordinates of at least one residue of a Polo-box domain with a ligand; (b) determining one or more moieties in the ligand to be modified; where the modified ligand maintains the ability to bind the Polo-box domain; and (c) modifying the ligand based on the determination. In one embodiment, the method further involves crystallizing a Polo-box domain with a ligand. In another embodiment, the ligand specifically binds the Polo-box domain. In another embodiment, the modification increases the affinity of the ligand for the Polo-box domain. In

another embodiment, the modification increases the solubility of the ligand. In another embodiment, the modification increases the half-life of the ligand *in vivo*.

In another aspect, the invention provides a method for manufacturing a Polo-box domain ligand, the method involves manufacturing a ligand that binds a Polo-box domain; where the ligand is designed or selected based on information obtained using a model of the atomic coordinates of at least a portion of the Polo-box domain.

In another aspect, the invention provides a method of evaluating the ability of a candidate ligand to bind a Polo-box domain, the method involves the steps of: (a) generating a three-dimensional structure of a Polo-box domain having at least one atomic coordinate, or surrogate thereof, from Table 5 for each of the following residues: His-538, Lys-540, Trp-414, or Leu-491 or atomic coordinates that have a root mean square deviation from the coordinates of less than 1, 2, 3, 4, or 5 Å; and (b) employing a means to measure the interaction between the candidate ligand and the Polo-box domain.

In another aspect, the invention provides a method of identifying a candidate ligand for a Polo-box domain, the method involves the steps of: (a) generating a three-dimensional pharmacophore model of Polo-box domain ligands using a computer of a previous aspect; and (b) selecting a candidate ligand satisfying the criteria of the pharmacophore model. In various embodiments, of any previous aspect, the method further involves determining the ability of the candidate ligand to bind the Polo-box domain *in vitro* or *in vivo*. In other embodiments, the method further involves determining the ability of the candidate ligand to alter the enzymatic activity of the Polo-box domain *in vitro* or *in vivo*. In other embodiments, the three-dimensional structure further comprises the hydrogen atoms of residues His-538, Lys-540, Trp-414, or Leu-491.

In various embodiments of the above aspects, the coordinate is for a heteroatom, or a side-chain atom, or a side-chain and a heteroatom. In other embodiments, the memory stores at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 coordinates

or surrogates thereof for His-538; at least 1, 2, 3, 4, 5, 6, 7, 8, or 9 coordinates or surrogates thereof for Lys-540, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 coordinates or surrogates thereof for Trp-414; or at least 1, 1, 2, 3, 4, 5, 6, 7, or 8 coordinates or surrogates thereof for Leu-491. In other embodiments, the coordinate is any one or all of the atomic coordinates in Table 5. In other embodiments of the previous aspect, the coordinates are for any residue required for the biological activity of a Polo box domain, or for binding a phosphopeptide or peptide mimetic. In other embodiments of any of the above aspects, root mean square deviation of the coordinates of less than 1, 2, 3, 4, 5, 6, or 7 Å.

In another aspect, the invention features a crystal of a Polo-like kinase complex containing a Polo-box domain bound to a phosphopeptide. In one embodiment, the ~~the~~ Polo-like kinase is Plk-1 (SEQ ID NO: 1). In another embodiment, the Plk-1 comprises at least amino acids 1-603 of SEQ ID NO:1. In another embodiment, the Plk-1 comprises at least amino acids 95-603. In another embodiment, the Plk-1 comprises at least amino acids 326-603. In another embodiment, the Plk-1 comprises at least amino acids 367-603. In another embodiment, the phosphopeptide comprises the amino acid sequence [Pro/Phe]-[ϕ/Pro]-[ϕ/Ala_{Cdc5p}/Gln_{Plk2}]-[Thr/Gln/His/Met]-Ser-[pThr/pSer]-[Pro/X] (SEQ ID NO: 2), where ϕ represents hydrophobic amino acids. In another embodiment, the phosphopeptide comprises the amino acid sequence MAGPMQ-S-**pT**-P-LNGAKK (SEQ ID NO: 3). In another embodiment, the Polo-like kinase is Plk-2 (SEQ ID NO: 4). In another embodiment, the Polo-like kinase is Plk-3 (SEQ ID NO: 5).

In another aspect, the invention provides a method of obtaining a structural model of a Polo-box domain of interest, the method involves homology modeling using at least a portion of the atomic coordinates in Table 5 and at least a portion of the amino acid sequence of the Polo-box domain of interest, thereby generating a model of the Polo-box domain of interest.

In another aspect, the invention provides a method of determining the three-dimensional structure of a Polo-box domain/phosphopeptide complex of interest, the method involves the steps of: (a) crystallizing the Polo-box domain/phosphopeptide complex of interest; (b) generating an X-ray diffraction pattern from the crystallized Polo-box domain of interest; and (c) applying at least a portion of the atomic coordinates in Table 5 to the diffraction pattern to generate a three-dimensional electron density map of at least a portion of the Polo-box domain/phosphopeptide complex of interest.

In another aspect, the invention features an isolated, less than full-length fragment of Polo-box domain containing residues 367-603 of human Plk-1 Polo-box domain) in complex with a phosphopeptide containing S-[pS/pT]-P/X, where X is any amino acid.

In another aspect, the invention features an isolated, less than full-length fragment of Polo-box domain containing residues residues 500-685 of human Plk-2 Polo-box domain in complex with a phosphopeptide containing S-[pS/pT]-P/X, where X is any amino acid.

In another aspect, the invention features an isolated, less than full-length fragment of Polo-box domain containing residues residues 421-607 of human Plk-3 Polo-box domain in complex with a phosphopeptide containing S-[pS/pT]-P/X, where X is any amino acid.

In another aspect, the invention features an isolated Polo-box domain protein or fragment thereof containing a mutation, where the mutation is (a) a mutation that enhances the ability of Polo-box domain to crystallize; (b) a mutation of a residue that is otherwise post-translationally modified in an organism used for recombinant expression; (c) a mutation of the NH2- or COOH-terminal residue of Polo-box domain; (d) a mutation that increases or decreases the affinity of a Polo-box domain for a phosphopeptide; or (e) a mutation that alters the folding of Polo-box domain. In one embodiment, the PBD further comprises a

mutation at His-538, Lys-540, Trp-414, or Leu-491. In other embodiments, the nucleic acid encodes a protein of any previous aspect.

In another aspect, the invention features a phosphopeptide containing the amino acid sequence [Pro/Phe]-[ϕ/Pro]-[ϕ/Ala_{Cdc5p}/Gln_{Plk2}]-[Thr/Gln/His/Met]-Ser-[pThr/pSer]-[Pro/X] (SEQ ID NO: 2), where ϕ represents hydrophobic amino acids. In one embodiment, the phosphopeptide comprises Pro-Met-Gln-Ser-pThr-Pro-Leu (SEQ ID NO: 6), where the phosphopeptide binds human Plk-1.

In another aspect, the invention features a phosphopeptide containing the amino acid sequence,

P-3	P-2	P-1	P0
Met	Ala	Ser	pSer
Tyr	His	Ala	pThr
Phe	Met	Gly	
Ile	Thr	Thr	
Leu	Phe		
His	Gln		
Lys			

;

(SEQ ID NO: 7), where pSer and pThr are phosphorylated serine and phosphorylated threonine, and where the amino acids designated in P-3, P-2, or P1 may be natural or unnatural amino acids. In one embodiment, the phosphopeptide of the previous aspect further contains the amino acid sequence,

	P-4	P-3	P-2	P-1	P0	P+1	
X_1aa		Met	Ala	Ser	pSer	Pro	X_2aa
		Tyr	His	Ala	pThr	Met	
		Phe	Met	Gly		Asn	
		Ile	Thr	Thr			
		Leu	Phe				
		His	Gln				
		Lys					

;

(SEQ ID NO: 8), where X_1aa and X_2aa are any amino acids and where pSer and pThr are phosphorylated serine and phosphorylated threonine. In another

embodiment, the X_1aa is proline and where X_2aa is any amino acid. In another embodiment, the X_1aa is any amino acid and where X_2aa is alanine, leucine, valine, isoleucine, phenylalanine, tyrosine, and tryptophan. In another embodiment, the X_2aa is leucine. In another embodiment, the amino acid at position P-3 is methionine. In another embodiment, the amino acid at position P-2 is glutamine. In another embodiment, the amino acid at position P-1 is serine. In another embodiment, the amino acid at position P0 is phosphorylated serine. In another embodiment, the amino acid at position P0 is phosphorylated threonine. In another embodiment, the amino acid at position P+1 is proline. In another embodiment, the amino acid sequence is Met-Gln-Ser-pThr-Pro-Leu or Met-Gln-Ser-pSer-Pro-Leu (SEQ ID NO: 9), where X_1aa is any amino acid and pThr is phosphorylated threonine and pSer is phosphorylated serine. In another embodiment, the phosphopeptide does not exceed 25 amino acids residues. In another embodiment, the phosphopeptide does not exceed 15 amino acids residues. In another embodiment, the phosphopeptide does not exceed 10 amino acids residues.

In another aspect, the invention features a pharmaceutical composition containing a therapeutic effective dose of any of the phosphopeptides of the previous aspects and a pharmaceutically acceptable excipient, where the pharmaceutical composition is useful for the treatment of a disorder characterized by inappropriate cell cycle regulation. In one embodiment, the cellular proliferative disorder is a neoplasm. In another embodiment, the composition further comprises a second chemotherapeutic agent. In another embodiment, the second chemotherapeutic agent is selected from the group consisting of paclitaxel, gemcitabine, doxorubicin, vinblastine, etoposide, 5-fluorouracil, carboplatin, altretamine, aminoglutethimide, amsacrine, anastrozole, azacitidine, bleomycin, busulfan, carmustine, chlorambucil, 2-chlorodeoxyadenosine, cisplatin, colchicine, cyclophosphamide, cytarabine, cytoxan, dacarbazine, dactinomycin, daunorubicin, docetaxel, estramustine phosphate, floxuridine, fludarabine, gentuzumab,

hexamethylmelamine, hydroxyurea, ifosfamide, imatinib, interferon, irinotecan, lomustine, mechlorethamine, melphalen, 6-mercaptopurine, methotrexate, mitomycin, mitotane, mitoxantrone, pentostatin, procarbazine, alemtuzumab, rituximab, streptozocin, tamoxifen, temozolomide, teniposide, 6-thioguanine, topotecan, trastuzumab, vincristine, vindesine, rofecoxib, celecoxib, etodolac and vinorelbine.

In another aspect, the invention features a method for treating or inhibiting a cellular proliferative disorder in a patient, the method involves administering a pharmaceutical composition of the phosphopeptide of a previous aspect, where the phosphopeptide is in an amount sufficient to treat or inhibit the cellular proliferative disorder in the patient. In one embodiment, method includes administering a second chemotherapeutic agent, the phosphopeptide and the chemotherapeutic agent are in amounts sufficient to treat or inhibit the cellular proliferative disorder in the patient, and where the chemotherapeutic agent is administered simultaneously or within 1, 2, 3, 5, 7, 10, 14, or 28 days of administering the phosphopeptide. In another embodiment, the second chemotherapeutic agent is selected from the group consisting of paclitaxel, gemcitabine, doxorubicin, vinblastine, etoposide, 5-fluorouracil, carboplatin, altretamine, aminoglutethimide, amsacrine, anastrozole, azacitidine, bleomycin, busulfan, carmustine, chlorambucil, 2-chlorodeoxyadenosine, cisplatin, colchicine, cyclophosphamide, cytarabine, cytoxan, dacarbazine, dactinomycin, daunorubicin, docetaxel, estramustine phosphate, floxuridine, fludarabine, gentuzumab, hexamethylmelamine, hydroxyurea, ifosfamide, imatinib, interferon, irinotecan, lomustine, mechlorethamine, melphalen, 6-mercaptopurine, methotrexate, mitomycin, mitotane, mitoxantrone, pentostatin, procarbazine, alemtuzumab, rituximab, streptozocin, tamoxifen, temozolomide, teniposide, 6-thioguanine, topotecan, trastuzumab, vincristine, vindesine, rofecoxib, celecoxib, etodolac and vinorelbine, or any other chemotherapeutic known in the art. In other embodiments, the cellular proliferative disorder is a neoplasm.

In another aspect, the invention features a method for identifying a peptidomimetic compound that modulates Polo-like kinase biological activity, the method involves the steps of: a) contacting the phosphopeptide of a previous aspect and a Polo-box domain (PBD) polypeptide to form a complex between the phosphopeptide and the PBD; b) contacting the complex with a candidate compound; and c) measuring the displacement of the phosphopeptide from the PBD, where the displacement of the phosphopeptide from the PBD indicates that the candidate compound is a peptidomimetic compound that modulates Polo-like kinase biological activity.

In another aspect, the invention provides a method for identifying a peptidomimetic compound that modulates Polo-like kinase biological activity, the method involves the steps of: a) contacting the phosphopeptide of a previous aspect and a PBD in the presence of a candidate compound; and b) measuring binding of the phosphopeptide and the PBD, where a reduction in the amount of binding relative to the amount of binding of the phosphopeptide and the polypeptide in the absence of the candidate compound indicates that the candidate compound is a peptidomimetic compound that modulates Polo-like kinase biological activity. In one embodiment, the phosphopeptide or the PBD is detectably labeled. In another embodiment, the phosphopeptide and the PBD are differentially labeled. In another embodiment, the PBD is selected from a group consisting of the PBDs of Cdc5, Plo-1, Polo, Plx-1, Plx-2, Plx-3, Plk-1, Prk/Fnk, Snk, and Cnk. In another embodiment, the PBD is Plk-1 PBD. In another embodiment, the Plk-1 PBD is human Plk-1 PBD.

In another aspect, the invention provides a method for identifying a binding pair consisting of a peptide and a peptide-binding domain, the method involes the steps of: a) providing a biased peptide library containing a collection of peptides fixed to a solid support, each peptide having at least two known amino acid residues whose position is invariant; b) providing a pooled cDNA library, where the cDNA library is positioned for protein expression; c) expressing the pooled

cDNA library in the presence of a detectable label; d) contacting the peptide library and the expressed cDNA library; and e) detecting a peptide and peptide-binding domain interaction, where an interaction identifies a peptide and peptide-binding domain binding pair. In one embodiment, the biased peptide library is covalently bound to a solid support. In another embodiment, the biased peptide library is noncovalently bound to a solid support. In another embodiment, the peptide is a phosphopeptide and the peptide binding domain is a phosphopeptide binding domain.

In another aspect, the invention provides a method for identifying a binding pair containing a phosphopeptide and a phosphopeptide binding domain, the method involves the steps of: a) providing a biased phosphopeptide library, containing a collection of peptides fixed to a solid support, each peptide having at least two known amino acid residues whose position is invariant; where each phosphopeptide is covalently linked to a biotin group at the amino terminus; b) providing a pooled cDNA library, where the pooled cDNA library is positioned for protein expression; c) expressing the pooled cDNA library in the presence of a detectable label; d) contacting the phosphopeptide library and the expressed cDNA library; and e) detecting a phosphopeptide and the phosphopeptide binding domain interaction, where the presence of an interaction identifies a phosphopeptide and phosphopeptide binding domain. In one embodiment, method further comprises the steps of f) providing a non-phosphorylated peptide of step a), and g) detecting a peptide and phosphopeptide-binding domain interaction, where the absence of an interaction indicates the phosphopeptide and phosphopeptide binding domain interaction is authentic.

In another aspect, the invention provides a method for identifying a binding pair consisting of a peptide and a peptide-binding domain; the method involves the steps of: a) providing a biased peptide library containing a collection of peptides fixed to a solid support, each peptide having at least two known amino acid residues whose position is invariant; b) contacting the biased peptide library with a

detectably labeled peptide library; and c) detecting a biased peptide and detectably labeled peptide interaction, where an interaction identifies a peptide and peptide-binding domain binding pair.

In another aspect, the invention features a method to identify phosphopeptide-binding modules, the method involves the steps of: (a) providing an immobilized phosphopeptide library and an immobilized peptide library; (b) contacting the libraries with a polypeptide or polypeptide fragment; and (c) detecting preferential binding, where preferential binding to the phosphopeptide library in comparison to the peptide library identifies the polypeptide or polypeptide fragment as a phosphopeptide binding module.

In another aspect, the invention provides a method to identify non-phosphopeptide-binding modules, the method involves the steps of: (a) providing an immobilized degenerate phosphopeptide library and an immobilized peptide library; (b) contacting the libraries with a polypeptide or polypeptide fragment; and (c) detecting preferential binding, where preferential binding to the peptide library in comparison to the phosphopeptide library identifies the polypeptide or polypeptide fragment as a non-phosphopeptide binding module.

In another aspect, the invention provides a method to identify phosphopeptide-binding modules in the DNA damage response pathway, the method involves the steps of: (a) providing an immobilized pSer or pThr degenerate phosphopeptide library and an immobilized Ser or Thr peptide library; (b) contacting the libraries with a polypeptide or polypeptide fragment; and (c) detecting differential binding, where preferential binding to the phosphopeptide library in comparison to the peptide library identifies the polypeptide or polypeptide fragment as a phosphopeptide binding module. In one embodiment, the phosphopeptide or peptide libraries do not have the amino acids Arg, Lys, or His in a degenerate position in the libraries. In another embodiment, the polypeptides or polypeptide fragments are *in vitro* translated (IVT) polypeptides.

In another aspect, the invention features a degenerate phosphopeptide containing a pSer or pThr that binds a BRCT domain. In one embodiment, the phosphopeptide further comprises an aromatic or aliphatic residue in the pSer or pThr +3 position; aromatic or aliphatic residues in the pSer or pThr +3 or +5 positions; a Gln or an aromatic or an aliphatic residue in the +1 position; or the amino acid sequence Y-D-I-(pSer or pThr)-Q-V-F-P-F (SEQ ID NO: 10).

In another aspect, the invention features a phosphopeptide binding module containing a BRCT tandem domain. In one embodiment, the BRCT tandem domain comprises at least 100 amino acids of the 3rd and 4th BRCT domains of PTIP. In another embodiment, the BRCT pair comprises at least 100 amino acids of the BRCT domains of BRCA1. In another embodiment, the tandem domain functions as a single module in phosphopeptide binding.

In another aspect, the invention features an isolated fragment (e.g, 50, 100, 150, 200, 250, or 300 amino acids) of tandem BRCT domains of PTIP or BRCA1 in complex with a phosphopeptide containing a pSer or pThr amino acid.

In another aspect, the invention features a complex containing a tandem BRCT phosphopeptide binding module and a phosphopeptide containing a pSer or pThr. In one embodiment, the tandem BRCT phosphopeptide binding module is a fragment of PTIP in complex with a phosphopeptide. In another embodiment, the phosphopeptide further comprises an aromatic or aliphatic residue in the (pSer or pThr)+3 position; an aromatic or aliphatic residues in the (pSer or pThr)+3 or +5 positions a Gln, or an aromatic or aliphatic residue in the +1 position; or the amino acid sequence Y-D-I-(pSer or pThr)-Q-V-F-P-F (SEQ ID NO: 10). In another aspect, the invention provides a method for identifying a candidate compound for the treatment or prevention of a neoplasia, the method containing detecting binding of the phosphopeptide binding module to a phosphopeptide in the presence of the candidate compound, where a candidate compound that modulates the binding is a compound useful for the treatment or prevention of a neoplasia. In one embodiment, binding is detected using an immunological assay, an enzymatic

assay, or a radioimmunoassay. In another embodiment, the phosphopeptide binding module or fragment thereof is an isolated phosphopeptide binding module. In another embodiment, the phosphopeptide binding module or fragment thereof is an isolated phosphopeptide containing a pSer or pThr. In one embodiment, phosphopeptide is fixed to a solid support. In another embodiment, the phosphopeptide binding module is a tandem BRCT binding domain. In another embodiment, the phosphopeptide binding module is fixed to a solid support. In another embodiment, the binding is assayed using an immunological assay, an enzymatic assay, or a radioimmunoassay. In another embodiment, the candidate compound is preincubated with the phosphopeptide binding module. In another embodiment, the candidate compound is preincubated with the phosphopeptide. In another embodiment, the phosphopeptide binding module and the phosphopeptide form a complex prior to being contacted with the candidate compound. In another embodiment, the candidate compound, the phosphopeptide and the phosphopeptide binding module are contacted concurrently.

In another aspect, the invention features a method for identifying a candidate compound useful in treating or preventing a neoplasia in a subject, the method involves:(a) providing a cell expressing a phosphopeptide binding module or fragment thereof and a phosphopeptide containing a pSer or pThr; (b) contacting the cell with a candidate compound; and (c) comparing binding of the phosphopeptide binding module and the phosphopeptide in the cell contacted with the candidate compound to the binding in a control cell, where a modulation of the binding identifies the candidate compound as a compound useful to treat or prevent a neoplasia in a subject. In one embodiment, phosphopeptide binding moduleand the phosphopeptide are expressed in a prokaryotic or a eukaryotic cell in vitro. In another embodiment, the phosphopeptide binding module is expressed endogenously by the cell. In another embodiment, the phosphopeptide binding module is expressed as a recombinant protein. In another embodiment, the cell is a neoplastic cell. In another embodiment, the neoplastic cell is a mammalian cell.

In another embodiment, the neoplastic cell is a human cell. In another embodiment, the candidate compound decreases the affinity of the binding.

In another aspect, the invention features a pharmaceutical composition containing (i) a phosphopeptide containing a pSer or pThr and (ii) a pharmaceutically acceptable carrier, where the phosphopeptide is present in amounts that, when administered to a subject, ameliorates a neoplastic disease. In one embodiment, the compositions comprises a second chemotherapeutic agent. In another embodiment, the second chemotherapeutic agent is selected from the group consisting of paclitaxel, gemcitabine, doxorubicin, vinblastine, etoposide, 5-fluorouracil, carboplatin, altretamine, aminoglutethimide, amsacrine, anastrozole, azacitidine, bleomycin, busulfan, carmustine, chlorambucil, 2-chlorodeoxyadenosine, cisplatin, colchicine, cyclophosphamide, cytarabine, cytoxan, dacarbazine, dactinomycin, daunorubicin, docetaxel, estramustine phosphate, floxuridine, fludarabine, gentuzumab, hexamethylmelamine, hydroxyurea, ifosfamide, imatinib, interferon, irinotecan, lomustine, mechlorethamine, melphalen, 6-mercaptopurine, methotrexate, mitomycin, mitotane, mitoxantrone, pentostatin, procarbazine, alemtuzumab, rituximab, streptozocin, tamoxifen, temozolomide, teniposide, 6-thioguanine, topotecan, trastuzumab, vincristine, vindesine, rofecoxib, celecoxib, etodolac and vinorelbine.

In another aspect, the invention provides a method for treating or inhibiting a cellular proliferative disorder in a patient, the method involves administering a pharmaceutical composition of the phosphopeptide of a previous aspect, where the phosphopeptide is in an amount sufficient to treat or inhibit the cellular proliferative disorder in the patient. In one embodiment, the method includes administering a second chemotherapeutic agent, the phosphopeptide and the chemotherapeutic agent are in amounts sufficient to treat or inhibit the cellular proliferative disorder in the patient, and where the chemotherapeutic agent is administered simultaneously or within fourteen days of administering the

phosphopeptide. In another embodiment, the second chemotherapeutic agent is selected from the group consisting of paclitaxel, gemcitabine, doxorubicin, vinblastine, etoposide, 5-fluorouracil, carboplatin, altretamine, aminoglutethimide, amsacrine, anastrozole, azacitidine, bleomycin, busulfan, carmustine, chlorambucil, 2-chlorodeoxyadenosine, cisplatin, colchicine, cyclophosphamide, cytarabine, cytoxan, dacarbazine, dactinomycin, daunorubicin, docetaxel, estramustine phosphate, floxuridine, fludarabine, gentuzumab, hexamethylmelamine, hydroxyurea, ifosfamide, imatinib, interferon, irinotecan, lomustine, mechlorethamine, melphalen, 6-mercaptopurine, methotrexate, mitomycin, mitotane, mitoxantrone, pentostatin, procarbazine, alemtuzumab, rituximab, streptozocin, tamoxifen, temozolomide, teniposide, 6-thioguanine, topotecan, trastuzumab, vincristine, vindesine, rofecoxib, celecoxib, etodolac and vinorelbine. In another embodiment, the cellular proliferative disorder is a neoplasm.

In another aspect, the invention features a method for identifying a peptidomimetic compound that modulates BRCT biological activity, the method involves the steps of: a) contacting the phosphopeptide of claim a previous aspect and a BRCT binding domain domain polypeptide to form a complex between the phosphopeptide and the BRCT; b) contacting the complex with a candidate compound; and c) measuring the displacement of the phosphopeptide from the BRCT binding domain, where the displacement of the phosphopeptide from the BRCT binding domain indicates that the candidate compound is a peptidomimetic compound that modulates BRCT binding domain biological activity.

In another aspect, the invention features a method for identifying a peptidomimetic compound that modulates BRCT binding domain biological activity, the method involves the steps of: a) contacting the phosphopeptide of a previous aspect and a BRCT binding domain in the presence of a candidate compound; and b) measuring binding of the phosphopeptide and the BRCT binding domain, where a reduction in the amount of binding relative to the amount

of binding of the phosphopeptide and the polypeptide in the absence of the candidate compound indicates that the candidate compound is a peptidomimetic compound that modulates BRCT binding domain biological activity. In one embodiment, the phosphopeptide or the BRCT binding domain is detectably labeled. In another embodiment, the phosphopeptide and the BRCT binding domain are differentially labeled. In other embodiments, the BRCT binding domain is BRCA1 or PTIP. In another embodiment, the BRCT binding domain is of human BRCA1. In one embodiment, BRCT binding domain is of human PTIP.

In another aspect, the invention features a kit containing (i) a small molecule that binds a BRCT binding domain and (ii) instructions for administering the small molecule to a patient diagnosed with or having a propensity to develop a neoplasia. In one embodiment, the kit further comprises a second chemotherapeutic compound.

In another aspect, the invention features a method of assessing a patient as having, or having a propensity to develop, a neoplasia, the method involves determining the level of expression of an a BRCT binding domain nucleic acid molecule or polypeptide in a patient sample, where an increased level of expression relative to the level of expression in a control sample, indicates that the patient has or has a propensity to develop a neoplasia. In one embodiment, the patient sample is a blood or tissue sample. In another embodiment, the method comprises determining the level of expression of the BRCT binding domain nucleic acid molecule. In another embodiment, the method comprises determining the level of expression of the a BRCT binding domain polypeptide. In another embodiment, the level of expression is determined in an immunological assay. In another embodiment, the method is used to diagnose a patient as having neoplasia.

In another aspect, the invention features a method to identify a peptide-binding module, the method involves the steps of: (a) providing an immobilized modified peptide library and an immobilized peptide library; (b) contacting the libraries with a polypeptide or polypeptide fragment; and (c) detecting preferential

binding, where preferential binding to the modified peptide library in comparison to the peptide library identifies the polypeptide or polypeptide fragment as a modified peptide binding module.

In another aspect, the invention features a method for identifying a binding pair consisting of a modified peptide and a peptide-binding domain, the method involves the steps of: a) providing a biased peptide library containing a collection of modified peptides fixed to a solid support, each peptide having one amino acid residues whose position is invariant; b) providing a pooled cDNA library, where the cDNA library is positioned for protein expression; c) expressing the pooled cDNA library in the presence of a detectable label; d) contacting the peptide library and the expressed cDNA library; and e) detecting a modified peptide and peptide-binding domain interaction, where an interaction identifies a modified peptide and peptide-binding domain binding pair. In one embodiment, the amino acid contains a modification that is natural or unnatural. In another embodiment, the modification is selected from the group consisting of methylation, acetylation, ubiquitination, glycosylation, sumolation, or arsenylation, or any other modification known to the skilled artisan.

In various embodiments of any of the above aspects, the peptide includes unnatural amino acids as described herein.

By "analog" is meant a molecule that is not identical but has analogous features. For example, a peptide analog retains the biological activity of a corresponding naturally-occurring peptide, while having certain biochemical modifications that enhance the analogs function relative to a naturally occurring peptide. Such biochemical modifications might increase the analogs protease resistance, membrane permeability, or half-life, without altering, for example, ligand binding. An analog can include a non-natural amino acid.

In another example, a nucleic acid analog retains the ability to hybridize to a naturally-occurring corresponding nucleic acid sequence, while having certain biochemical modifications that enhance the analogs function relative to a

naturally-occurring nucleic acid. In some nucleic acid analogs the sugar and/or the internucleoside linkage, i.e., the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. Peptide and nucleic acid modifications may be achieved by any of the techniques known in the art for derivatization of peptides or nucleic acids into fragments, analogs, or derivatives thereof. Such terms and in particular, "analog", also specifically include peptide, non-peptide, peptide/nucleic acid hybrid molecules, small molecules and other compounds that function as Polo-like kinase nucleic acid or peptide mimics.

By "apoptosis" is meant the process of cell death where a dying cell displays at least one of a set of well-characterized biological hallmarks, including cell membrane blebbing, cell soma shrinkage, chromatin condensation, or DNA laddering.

By "biased phosphopeptide library" is meant a phosphoserine, phosphothreonine, and/or phosphotyrosine degenerate peptide library, wherein specific amino acid residues of the phosphopeptide are fixed so as to be expressed in all phosphopeptides in the specific library. For instance, a biased phosphopeptide library can be synthesized to contain the core sequence Ser-pSer-Pro or Ser-pThr-Pro. In a desirable embodiment, the amino acid residue adjacent to the phosphoserine, phosphothreonine, or phosphotyrosine residue is fixed.

By an "amino acid fragment" is meant an amino acid residue that has been incorporated into a peptide chain via its alpha carboxyl, its alpha nitrogen, or both. A terminal amino acid is any natural or unnatural amino acid residue at the amino-terminus or the carboxy-terminus. An internal amino acid is any natural or unnatural amino acid residue that is not a terminal amino acid.

As used herein, the terms "alkyl" and the prefix "alk-" are inclusive of both straight chain and branched chain groups and of cyclic groups, i.e., cycloalkyl and cycloalkenyl groups. Cyclic groups can be monocyclic or polycyclic and

preferably have from 3 to 8 ring carbon atoms, inclusive. Exemplary cyclic groups include cyclopropyl, cyclopentyl, cyclohexyl, and adamantyl groups.

By "aromatic residue" is meant an aromatic group having a ring system with conjugated π electrons (e.g., phenyl or imidazole). The ring of the aryl group is preferably 5 to 6 atoms. The aromatic ring may be exclusively composed of carbon atoms or may be composed of a mixture of carbon atoms and heteroatoms. Preferred heteroatoms include nitrogen, oxygen, sulfur, and phosphorous. Aryl groups may optionally include monocyclic, bicyclic, or tricyclic rings, where each ring has preferably five or six members. The aryl group may be substituted or unsubstituted. Exemplary substituents include alkyl, hydroxyl, alkoxy, aryloxy, sulfhydryl, alkylthio, arylthio, halo, fluoroalkyl, carboxyl, carboxyalkyl, amino, aminoalkyl, monosubstituted amino, disubstituted amino, and quaternary amino groups.

By "aryl" is meant a carbocyclic aromatic ring or ring system. Unless otherwise specified, aryl groups are from 6 to 18 carbons. Examples of aryl groups include phenyl, naphthyl, biphenyl, fluorenyl, and indenyl groups.

By "heteroaryl" is meant an aromatic ring or ring system that contains at least one ring hetero-atom (e.g., O, S, N). Unless otherwise specified, heteroaryl groups are from 1 to 9 carbons.. Heteroaryl groups include furanyl, thienyl, pyrrolyl, imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, triazolyl, oxadiazolyl, oxatriazolyl, pyridyl, pyridazyl, pyrimidyl, pyrazyl, triazyl, benzofuranyl, isobenzofuranyl, benzothienyl, indole, indazolyl, indolizinyl, benzisoxazolyl, quinolinyl, isoquinolinyl, cinnolinyl, quinazolinyl, naphtyridinyl, phthalazinyl, phenanthrolinyl, purinyl, and carbazolyl groups.

By "heterocycle" is meant a non-aromatic ring or ring system that contains at least one ring heteroatom (e.g., O, S, N). Unless otherwise specified, heterocyclic groups are from 1 to 9 carbons. Heterocyclic groups include, for example, dihydropyrrolyl, tetrahydropyrrolyl, piperazinyl, pyranyl,

dihydropyranyl, tetrahydropyranyl, tetrahydrofuranyl, dihydrothiophene, tetrahydrothiophene, and morpholinyl groups.

By "halide" or "halogen" or "halo" is meant bromine, chlorine, iodine, or fluorine.

The aryl, heteroaryl, and heterocyclyl groups may be unsubstituted or substituted by one or more substituents selected from the group consisting of C_{1-5} alkyl, hydroxy, halo, nitro, C_{1-5} alkoxy, C_{1-5} alkylthio, trihalomethyl, C_{1-5} acyl, arylcarbonyl, heteroarylcarbonyl, nitrile, C_{1-5} alkoxycarbonyl, oxo, arylalkyl (wherein the alkyl group has from 1 to 5 carbon atoms) and heteroarylalkyl (wherein the alkyl group has from 1 to 5 carbon atoms).

By "biased phosphopeptide library" is meant a phosphoserine, phosphothreonine, and/or phosphotyrosine degenerate peptide library, wherein specific amino acid residues of the phosphopeptide are fixed so as to be expressed in all phosphopeptides in the specific library. For instance, a biased phosphopeptide library can be synthesized to contain the core sequence Ser-pSer-Pro or Ser-pThr-Pro. In a desirable embodiment, the amino acid residue adjacent to the phosphoserine, phosphothreonine, or phosphotyrosine residue is fixed.

By an "amino acid fragment" is meant an amino acid residue that has been incorporated into a peptide chain via its alpha carboxyl, its alpha nitrogen, or both. A terminal amino acid is any natural or unnatural amino acid residue at the amino-terminus or the carboxy-terminus. An internal amino acid is any natural or unnatural amino acid residue that is not a terminal amino acid.

As used herein, the terms "alkyl" and the prefix "alk-" are inclusive of both straight chain and branched chain groups and of cyclic groups, i.e., cycloalkyl and cycloalkenyl groups. Cyclic groups can be monocyclic or polycyclic and preferably have from 3 to 8 ring carbon atoms, inclusive. Exemplary cyclic groups include cyclopropyl, cyclopentyl, cyclohexyl, and adamantyl groups.

By "aromatic residue" is meant an aromatic group having a ring system with conjugated π electrons (e.g., phenyl or imidazole). The ring of the aryl group

is preferably 5 to 6 atoms. The aromatic ring may be exclusively composed of carbon atoms or may be composed of a mixture of carbon atoms and heteroatoms. Preferred heteroatoms include nitrogen, oxygen, sulfur, and phosphorous. Aryl groups may optionally include monocyclic, bicyclic, or tricyclic rings, where each ring has preferably five or six members. The aryl group may be substituted or unsubstituted. Exemplary substituents include alkyl, hydroxyl, alkoxy, aryloxy, sulfhydryl, alkylthio, arylthio, halo, fluoroalkyl, carboxyl, carboxyalkyl, amino, aminoalkyl, monosubstituted amino, disubstituted amino, and quaternary amino groups.

By "aryl" is meant a carbocyclic aromatic ring or ring system. Unless otherwise specified, aryl groups are from 6 to 18 carbons. Examples of aryl groups include phenyl, naphthyl, biphenyl, fluorenyl, and indenyl groups.

By "BRCA1 nucleic acid" is meant a nucleic acid, or analog thereof, that encodes BRCA1 or is substantially identical to Gene Bank Accession No: 30039658 (SEQ ID NO: 11).

By "BRCA1 polypeptide" is meant a polypeptide, or analog thereof, substantially identical to BRCA1 Genbank Accession NO. 30039659 (SEQ ID NO: 12) and having BRCA1 biological activity.

By "BRCA1 biological activity" is meant function in a DNA damage response pathway or phosphopeptide binding.

By "BRCT nucleic acid is meant a nucleic acid, or nucleic acid analog, that encodes tandem BRCT domains. For example, a nucleic acid substantially identical to PTIP BC033781[21707457] (SEQ ID NO: 13), or NM_007349 (PAX transcription activation domain interacting protein 1 mRNA) (SEQ ID NO: 14) or Gene Bank Accession No: AY273801[30039658] (SEQ ID NO: 11).

By "tandem BRCT polypeptide is meant a protein having at least 2 tandem BRCT domains. For example, a protein substantially identical to AAH33781 (SEQ ID NO:15), NP_031375 (SEQ ID NO: 16), or Genbank Accession NO. 30039659 (SEQ ID NO: 12).

By "candidate compound" is meant any nucleic acid molecule, polypeptide, or other small molecule, that is assayed for its ability to alter gene or protein expression levels, or the biological activity of a gene or protein by employing one of the assay methods described herein. Candidate compounds include, for example, peptides, polypeptides, synthesized organic molecules, naturally occurring organic molecules, nucleic acid molecules, and components thereof.

By "detectably-labeled" is meant any means for marking and identifying the presence of a molecule, e.g., a PBD-interacting phosphopeptide, a PBD, a nucleic acid encoding the same, or a peptidomimetic small molecule. Methods for detectably-labeling a molecule are well known in the art and include, without limitation, radionuclides (e.g., with an isotope such as ^{32}P, ^{33}P, ^{125}I, or ^{35}S) and nonradioactive labeling (e.g., chemiluminescent labeling or fluorescein labeling).

If required, molecules can be differentially labeled using markers that can distinguish the presence of multiply distinct molecules. For example, a PBD domain-interacting phosphopeptide can be labeled with fluorescein and a PBD domain polypeptide can be labeled with Texas Red. The presence of the phosphopeptide can be monitored simultaneously with the presence of the PBD.

By "diseases or disorder characterized by inappropriate cell cycle control" is meant any pathological condition in which there is an abnormal increase or decrease in cell proliferation. Exemplary diseases or disorder characterized by inappropriate cell cycle control include cancer or neoplasms, inflammatory diseases, or hyperplasias (e.g. some forms of hypertension, prostatic hyperplasia).

By "disease or disorder characterized by inappropriate cell death" is meant any pathological condition in which there is an abnormal increase in apoptosis. Exemplary diseases or disorders characterized by inappropriate cell death include neurodegenerative diseases (e.g., Alzheimer's, Huntington's, and Parkinson's disease), cardiac disorders (e.g., congestive heart failure and myocardial infarction), diabetic retinopathy, and age-related macular degeneration.

By "fragment" is meant a portion of a protein (50, 100, 150, 175, 200, 300, or 400 amino acids) or nucleic acid (50, 100, 150, 175, 200, 300, or 400 nucleic acids) that is substantially identical to a reference protein or nucleic acid, and retains at least 50% or 75%, more preferably 80%, 90%, or 95%, or even 99% of the biological activity of the reference protein or nucleic acid using a molting assay as described herein.

By "heteroaryl" is meant an aromatic ring or ring system that contains at least one ring hetero-atom (e.g., O, S, N). Unless otherwise specified, heteroaryl groups are from 1 to 9 carbons.. Heteroaryl groups include furanyl, thienyl, pyrrolyl, imidazolyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, isothiazolyl, triazolyl, oxadiazolyl, oxatriazolyl, pyridyl, pyridazyl, pyrimidyl, pyrazyl, triazyl, benzofuranyl, isobenzofuranyl, benzothienyl, indole, indazolyl, indolizinyl, benzisoxazolyl, quinolinyl, isoquinolinyl, cinnolinyl, quinazolinyl, naphtyridinyl, phthalazinyl, phenanthrolinyl, purinyl, and carbazolyl groups.

By "heterocycle" is meant a non-aromatic ring or ring system that contains at least one ring heteroatom (e.g., O, S, N). Unless otherwise specified, heterocyclic groups are from 1 to 9 carbons. Heterocyclic groups include, for example, dihydropyrrolyl, tetrahydropyrrolyl, piperazinyl, pyranyl, dihydropyranyl, tetrahydropyranyl, tetrahydrofuranyl, dihydrothiophene, tetrahydrothiophene, and morpholinyl groups.

By "halide" or "halogen" or "halo" is meant bromine, chlorine, iodine, or fluorine.

The aryl, heteroaryl, and heterocyclyl groups may be unsubstituted or substituted by one or more substituents selected from the group consisting of C_{1-5} alkyl, hydroxy, halo, nitro, C_{1-5} alkoxy, C_{1-5} alkylthio, trihalomethyl, C_{1-5} acyl, arylcarbonyl, heteroarylcarbonyl, nitrile, C_{1-5} alkoxycarbonyl, oxo, arylalkyl (wherein the alkyl group has from 1 to 5 carbon atoms) and heteroarylalkyl (wherein the alkyl group has from 1 to 5 carbon atoms).

By "isolated polynucleotide" is meant a nucleic acid (e.g., a DNA) that is free of the genes which, in the naturally-occurring genome of the organism from which the nucleic acid molecule of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or that exists as a separate molecule (for example, a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. In addition, the term includes an RNA molecule which is transcribed from a DNA molecule, as well as a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

By "isolated polypeptide" is meant a polypeptide of the invention that has been separated from components which naturally accompany it. Typically, the polypeptide is isolated when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, a polypeptide of the invention. An isolated polypeptide of the invention may be obtained, for example, by extraction from a natural source, by expression of a recombinant nucleic acid encoding such a polypeptide; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, for example, column chromatography, polyacrylamide gel electrophoresis, or by HPLC analysis.

By "modulate" is meant a change, such as a decrease or increase. Desirably, the change is either an increase or a decrease of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95% in expression or biological activity, relative to a reference or to control expression or activity, for example the expression or biological activity of a naturally occurring Polo-like kinase.

By "neoplasia" is meant a disease characterized by the pathological proliferation of a cell or tissue and its subsequent migration to or invasion of other

tissues or organs. Neoplasia growth is typically uncontrolled and progressive, and occurs under conditions that would not elicit, or would cause cessation of, multiplication of normal cells. Neoplasias can affect a variety of cell types, tissues, or organs, including but not limited to an organ selected from the group consisting of bladder, bone, brain, breast, cartilage, glia, esophagus, fallopian tube, gallbladder, heart, intestines, kidney, liver, lung, lymph node, nervous tissue, ovaries, pancreas, prostate, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urogenital tract, ureter, urethra, uterus, and vagina, or a tissue or cell type thereof. Neoplasias include cancers, such as sarcomas, carcinomas, or plasmacytomas (e.g., acute lymphocytic leukemia, acute myelocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroleukemia, chronic leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia, polycythemia vera, lymphoma Hodgkin's disease, Waldenstrom's macroglobulinemia, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, nile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, uterine cancer, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodenriglioma, schwannoma, meningioma, melanoma, neuroblastoma, or retinoplastoma).

By "nucleic acid" is meant an oligomer or polymer of ribonucleic acid or deoxyribonucleic acid, or analog thereof. This term includes oligomers consisting of naturally occurring bases, sugars, and intersugar (backbone) linkages as well as oligomers having non-naturally occurring portions which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of properties such as, for example, enhanced cellular uptake and increased stability in the presence of nucleases.

Specific examples of some preferred nucleic acids envisioned for this invention may contain phosphorothioates, phosphotriesters, methyl phosphonates, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages. Most preferred are those with CH_2 –NH—O—CH_2, CH_2—N(CH_3)—O—CH_2, CH_2—O—N(CH_3)—CH_2, CH_2—N(CH_3)—N(CH_3)—CH_2 and O—N(CH_3)—CH_2—CH_2 backbones (where phosphodiester is O—P—O—CH_2). Also preferred are oligonucleotides having morpholino backbone structures (Summerton, J.E. and Weller, D.D., U.S. Pat. No: 5,034,506). In other preferred embodiments, such as the protein-nucleic acid (PNA) backbone, the phosphodiester backbone of the oligonucleotide may be replaced with a polyamide backbone, the bases being bound directly or indirectly to the aza nitrogen atoms of the polyamide backbone (P.E. Nielsen et al. *Science* 199: 254, 1997). Other preferred oligonucleotides may contain alkyl and halogen-substituted sugar moieties comprising one of the following at the 2' position: OH, SH, SCH_3, F, OCN, $O(CH_2)_nNH_2$ or $O(CH_2)_n$ CH_3, where n is from 1 to about 10; C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl or aralkyl; Cl; Br; CN; CF_3; OCF_3; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; $SOCH_3$; SO_2CH_3; ONO_2; NO_2; N_3; NH_2; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; substituted silyl; an RNA cleaving group; a conjugate; a reporter group; an intercalator; a group for improving the pharmacokinetic properties of an oligonucleotide; or a group for improving the pharmacodynamic properties of an oligonucleotide and other substituents having similar properties. Oligonucleotides

may also have sugar mimetics such as cyclobutyls in place of the pentofuranosyl group.

Other preferred embodiments may include at least one modified base form. Some specific examples of such modified bases include 2-(amino)adenine, 2-(methylamino)adenine, 2-(imidazolylalkyl)adenine, 2-(aminoalklyamino)adenine, or other heterosubstituted alkyladenines.

By "Pax2 *trans*-activation domain-interacting protein (PTIP) nucleic acid" is meant a nucleic acid, or analog thereof, substantially identical to Genebank Accession No:21707457 (SEQ ID NO: 13) or NM_007349 (SEQ ID NO: 14).

By "Pax2 *trans*-activation domain-interacting protein (PTIP)" is meant a polypeptide, or analog thereof, substantially identical to Genebank Accession No: AAH33781.1 (SEQ ID NO: 15) or NP_031375 (SEQ ID NO: 16), and having PTIP biological activity.

By "PTIP biological activity" is meant function in a DNA damage response pathway or phosphopeptide binding.

By "pharmaceutically acceptable excipient" is meant a carrier that is physiologically acceptable to the subject to which it is administered and that preserves the therapeutic properties of the compound with which it is administered. One exemplary pharmaceutically acceptable excipient is physiological saline. Other physiologically acceptable excipients and their formulations are known to one skilled in the art and described, for example, in "Remington: The Science and Practice of Pharmacy" (20th ed., ed. A.R. Gennaro AR., 2000, Lippincott Williams & Wilkins).

By a "peptidomimetic" is meant a compound that is capable of mimicking or antagonizing the biological actions of a natural parent peptide. A peptidomimetic may include non-peptidic structural elements, unnatural peptides, synthesized organic molecules, naturally occurring organic molecules, nucleic acid molecules, and components thereof. Identification of a peptidomimetic can be accomplished by screening methods incorporating a binding pair and identifying

compounds that displace the binding pair. Alternatively, a peptidomimetic can be designed *in silico*, by molecular modeling of a known protein-protein interaction, for example, the interaction of a phosphopeptide of the invention and a PBD. Desirably, the peptidomimetic will displace one member of a binding pair by occupying the same binding interface. More desirably the peptidomimetic will have a higher binding affinity to the binding interface.

By "Polo-like kinase (PLK) nucleic acid molecule" is meant a nucleic acid, or nucleic acid analog, that encodes a Polo-like kinase polypeptide. For example, a Plk-1 nucleic acid molecule is substantially identical to GenBank Accession Number X73458 (SEQ ID NO: 17) or NM_005030 (SEQ ID NO: 18); a Plk-2/SNK nucleic acid molecule is substantially identical to NM_006622 (SEQ ID NO: 19); a Plk-3 nucleic acid molecule is substantially identical to NM_004073 (SEQ ID NO: 20); a Plx-1 nucleotide sequence is substantially identical to GenBank Accession Number U58205 (SEQ ID NO: 21); and a Polo nucleic acid molecule is substantially identical to GenBank Accession Number AY095028 (SEQ ID NO: 22) or NM_079455 (SEQ ID NO: 23).

By a "Polo-like kinase" is meant a polypeptide substantially identical to a Polo-like kinase amino acid sequence, having serine/threonine kinase activity, and having at least one Polo-box domain consisting of 2 Polo-boxes. Exemplary Polo-like kinase polypeptides include, Plk-1 (GenBank Accession Number NP_005021, SEQ ID NO:1); Plk-2 (GenBank Accession Number NP_006613, SEQ ID NO:4); and Plk-3 (GenBank Accession Number NP_004064, SEQ ID NO:5). Additional Polo-like kinase polypeptides include GenBank Accession Numbers P53350 (SEQ ID NO: 24), and Q07832 (SEQ ID NO: 25).

Structurally, Polo or Polo-like kinases have a unique amino terminus followed by a serine/threonine kinase domain, a linker region, a Polo-box (PB1), a linker sequence, a second Polo-box (PB 2), and a small stretch of 12-20 amino acids at the carboxy terminus (see Figure 2A).

In desirable embodiments, Polo-like kinases include *Saccaromyces cereviseae*, Cdc5, *Schizosaccaromyces pombe*, Plo-1, *Drosophila melanogaster*, Polo, *Xenopus laevis*, Plx (Plx-1, -2, -3), and mammalian Plk-1, Prk/Fnk, Snk, and Cnk. The Polo-box is approximately 70 amino acids in length and is shown in Figure 2B (indicated by the bold lines).

By "Polo-like kinase biological activity" is meant any biological activity associated with Polo-like kinases, such as serine/threonine kinase activity. Other biological activities of Polo-like kinases include the localization of the kinase to the centrosomes, spindle apparatus, and microtubular organizing centers (MOCs).

By "polypeptide" is meant any chain of at least two naturally-occurring amino acids, or unnatural amino acids (e.g., those amino acids that do not occur in nature) regardless of post-translational modification (e.g., glycosylation or phosphorylation), constituting all or part of a naturally-occurring or unnatural polypeptide or peptide, as is described herein. Naturally occurring amino acids are any one of the following, alanine (A or Ala), cysteine (C or Cys), aspartic acid (D or Asp), glutamic acid (E or Glu), phenylalanine (F or Phe), glycine (G or Gly), histidine (H, or His), isoleucine (I or Ile), lysine (K or Lys), leucine (L or Leu), methionine (M or Met), asparagine (N or Asn), ornithine (O or Orn), proline (P or Pro), hydroxyproline (Hyp), glutamine (Q or Gln), arginine (R or Arg), serine (S or Ser), threonine (T or Thr), valine (V or Val), tryptophan (W or Trp), or tyrosine (Y or Tyr).

By "peptide" is meant any compound composed of amino acids, amino acid analogs, chemically bound together. In general, the amino acids are chemically bound together via amide linkages (CONH); however, the amino acids may be bound together by other chemical bonds known in the art. For example, the amino acids may be bound by amine linkages. Peptide as used herein includes oligomers of amino acids, amino acid analog, or small and large peptides, including polypeptides.

Polypeptides or derivatives thereof may be fused or attached to another protein or peptide, for example, as a Glutathione-S-Transferase (GST) fusion polypeptide. Other commonly employed fusion polypeptides include, but are not limited to, maltose-binding protein, *Staphylococcus aureus* protein A, Flag-Tag, HA-tag, green fluorescent proteins (e.g., eGFP, eYFP, eCFP, GFP, YFP, CFP), red fluorescent protein, polyhistidine (6xHis), and cellulose-binding protein.

By "phosphopeptide" or "phosphoprotein" means a peptide or protein in which one or more phosphate moieties are covalently linked to serine, threonine, tyrosine, aspartic acid, histidine amino acid residues, or amino acid analogs. A peptide can be phosphorylated to the extent of the number of serine, threonine, tyrosine, or histidine amino acid residues that is present. Desirably, a phosphopeptide is phosphorylated at 4 independent Ser/Thr/Tyr residues, at 3 independent Ser/Thr/Tyr residues, or at 2 independent Ser/Thr/Tyr residues. Most desirably, a phosphopeptide is phosphorylated at one Ser/Thr/Tyr residue regardless of the presence of multiple Ser, Thr, or Tyr residues.

Typically, a phosphopeptide is produced by expression in a prokaryotic or eukaryotic cell under appropriate conditions or in translation extracts where the peptide is subsequently isolated, and phosphorylated using an appropriate kinase. Alternatively, a phosphopeptide may be synthesized by standard chemical methods, for example, using N-α-FMOC-protected amino acids (including appropriate phosphoamino acids). In a desired embodiment, the use of non-hydrolysable phosphate analogs can be incorporated to produce non-hydrolysable phosphopeptides (Jenkins et al., *J. Am. Chem. Soc.*, 124:6584-6593, 2002; herein incorporated by reference). Such methods of protein synthesis are commonly used and practiced by standard methods in molecular biology and protein biochemistry (Ausubel *et al.*, Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, 1994, J. Sambrook and D. Russel, Molecular Cloning: A Laboratory Manual, 3rd Edition, Cold Spring Harbor Laboratory Press, Woodbury NY, 2000). Desirably, a phosphopeptide employed in the invention is generally not longer

than 100 amino acid residues in length, desirably less than 50 residues, more desirably less than 25 residues, 20 residues, 15 residues. Most desirably the phosphopeptide is 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid residues long.

By "substantially identical" is meant a polypeptide or nucleic acid exhibiting at least 75%, but preferably 85%, more preferably 90%, most preferably 95%, or even 99% identity to a reference amino acid or nucleic acid sequence. For polypeptides, the length of comparison sequences will generally be at least 35 amino acids, preferably at least 45 amino acids, more preferably at least 55 amino acids, and most preferably 70 amino acids. For nucleic acids, the length of comparison sequences will generally be at least 60 nucleotides, preferably at least 90 nucleotides, and more preferably at least 120 nucleotides.

Sequence identity is typically measured using sequence analysis software with the default parameters specified therein (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). This software matches similar sequences by assigning degrees of homology to various substitutions, deletions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine, valine, isoleucine, leucine, methionine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

By "unnatural amino acid" is meant an organic compound that has a structure similar to a natural amino acid, where it mimics the structure and reactivity of a natural amino acid. The unnatural amino acid as defined herein generally increases or enhances the properties of a peptide (e.g., selectivity, stability, binding affinity) when the unnatural amino acid is either substituted for a natural amino acid or incorporated into a peptide.

Unnatural amino acids and peptides including such amino acids are described in U.S. Patent No. 6,566,330 and 6,555,522.

Other features and advantages of the invention will be apparent from the following description of the desirable embodiments thereof, and from the claims.

Brief Description of the Drawings

The application file contains drawings executed in color (Figures 10, 11, 12, 14, and 21). Copies of this patent or patent application with color drawings will be provided by the Office upon request and payment of the necessary fee.

Figures 1A and 1B depict a novel phospho-motif-based library vs. library screen to identify phosphoserine/threonine binding domains. Figure 1A depicts a library of phosphothreonine-proline oriented phosphopeptides, biased toward the phosphorylation motifs for cyclin-dependent kinases and MAP kinases and toward the epitope of the monoclonal antibody MPM-2, and immobilized on Streptavidin beads. This library and its unphosphorylated counterpart were screened against 680 pools of *in vitro* translated ^{35}S-Met labeled proteins. pT denotes phosphothreonine. B represents a biased mixture of the amino acids P, L, I, V, F, M, W. Figure 1B is a set of four SDS-PAGE/autoradiographs. The WW-domain containing protein Pin1 and a fragment of the mitotic kinase Plk-1, denoted by asterisks, were isolated from two pools as clones that associated preferentially with the phosphorylated form of the immobilized peptide library. In each panel, the first lane shows 10% of the input radiolabeled protein pool, while the second and third lanes show binding of proteins within this pool to the phosphorylated and unphosphorylated immobilized libraries, respectively. Identification of Pin1 and Plk1 occurred through progressive subdivision of their respective pools to single clones (panels on right). Arrowheads indicate partial translation or proteolytic breakdown products of Plk1 that exhibit more dramatic phospho-discrimination than the full-length transcript of the isolated Plk1 fragment, suggesting that the full-length transcript likely contains a smaller discrete phospho-binding domain.

Figure 2A is a schematic diagram showing various C-terminal truncations of Plk-1, translated *in vitro*, and assayed for selective binding to the phosphorylated peptide library of Figure 1A over its unphosphorylated counterpart. The two shaded regions in the C-terminus of Plk-1 correspond to its polo boxes (PB1 and PB2) as defined by Pfam. Truncated constructs were designed according to boundaries of sequence homology within the polo-like kinase family rather than boundaries of the Pfam-delineated polo boxes. Clone 407-C6 is the fragment of Plk-1 isolated from the screen depicted in Figures 1A and B.

Figure 2B shows an amino acid sequence alignment of the C-terminal noncatalytic region of human Plk-1 (SEQ ID NO: 77), *Xenopus* Plx-1 (SEQ ID NO: 78), and *Drosophila* Polo (SEQ ID NO: 79). Bold lines indicate the designated polo boxes (PB1 and PB2) of Plk-1 as defined by Pfam.

Figures 3A-3D are histograms showing the binding ratios of the Plk-1 polo-box domain (PBD). The Polo-box Domain (PBD, residues 326-603) of Plk-1 was expressed as a GST fusion protein, immobilized on Glutathione-agarose beads, and incubated with phosphothreonine/serine-oriented degenerate peptide libraries consisting of the sequences MAXXXXpTPXXXXAKK (SEQ ID NO:~~11~~ 26) (3A), MAXXXXpSPXXXXAKK (SEQ ID NO:~~12~~ 27) (3B), MAXXXXSpTXXXXAKK (SEQ ID NO:~~13~~ 28) (3C), or MAXXXXSpSXXXXAKK (SEQ ID NO:~~14~~ 29) (3D) where X indicates all amino acids except Cys. Following extensive washing, bound peptides were eluted and sequenced. The bar graphs show the relative abundance of each amino acid at a given cycle of sequencing compared to its abundance in the starting peptide library mixture. The Plk-1 PBD selects for serine in the pThr/Ser-1 position strongly (5.9 or 8.1) and for proline in the pThr/Ser+1 position moderately (1.6 or 1.8).

Figure 3E is an autoradiograph. Pin1 (3E) shows an absolute requirement for proline in the pThr+1 position, whereas the PBD of Plk-1 does not. Full-length Pin1 and the PBD (residues 326-603) of Plk-1 were translated *in vitro* in the

presence of ^{35}S-methionine and tested for binding to four immobilized peptide libraries that differed by phosphorylation status and/or the presence of proline in the pThr+1 position.

pTP= biotin-ZGZGGAXXBXpTPXXXXAKKK (SEQ ID NO:~~15~~ 30),

TP= biotin-ZGZGGAXXBXTPXXXXAKKK (SEQ ID NO:~~16~~ 31),

pT= biotin-ZGZGGAXXXXpTXXXXXAKKK (SEQ ID NO:~~17~~ 32),

T= biotin-ZGZGGAXXXXTXXXXXAKKK (SEQ ID NO:~~18~~ 33),

where pT is phosphothreonine, Z indicates aminohexanoic acid, X denotes all amino acids except Cys, and B is a biased mixture of the amino acids P, L, I, V, F, M, W.

Figure 4A shows isothermal titration calorimetry results. These results show that Plk1 PBD binds its optimal phosphopeptide ligand with high affinity and high specificity.

Figure 4B is a table. Isothermal titration calorimetry (ITC) was used to determine binding constants (K_d) for the association of the Plk-1 PBD (residues 326-603) with its optimal phosphopeptide ligand and with nine mutated versions of this peptide. All observed binding stoichiometries were consistent with a 1:1 complex of PBD and phosphopeptide. N.D.B indicates no detectable binding by ITC for a Plk-1 PBD concentration of at least 150μM. pT, pS, and pY denote phosphothreonine, phosphoserine, and phosphotyrosine, respectively.

Figures 5A upper panel shows a FACS (fluorescence activated cell sorter) trace of human cells used in the pull-down assays shown below. The upper left panel shows the FACS profile of the cells arrested with aphidocolin in G1 (so the total DNA content is 1N where N = the normal amount of DNA in a diploid human cell) and verifies that the cells were in G1. The right trace shows the FACS profile of the cells arrested with nocadozole to trap them in G2/M, and shows that their DNA content is 2N, verifying that they are arrested in G2/M. Figures 5A (lower panel) and 5B are immunoblots showing that the Plk-1 PBD associates with mitotic phosphoproteins in HeLa cells. Lysates from HeLa cells,

arrested at interphase with aphidicolin or in G2/M with nocodazole, were incubated with GST, GST-Pin1, and the GST-Plk-1 PBD (residues 326-603; Figure 5A). Mitotic phosphoproteins co-precipitated with these GST fusions were detected by blotting with the pSer-Pro specific monoclonal antibody MPM-2. Interaction of the GST-Plk-1 PBD (residues 326-603) with mitotic phosphoproteins from nocodazole-arrested HeLa cells was disrupted by pre-incubation of GST-Plk-1 PBD with its optimal phosphopeptide ligand, MAGPMQ-S-**pT**-P-LNGAKK (SEQ ID NO:~~19~~ 2) (PoloBoxtide-optimal), but not with an unphosphorylated equivalent peptide, MAGPMQ-S-**T**-P-LNGAKK (SEQ ID NO:~~20~~ 34) (PoloBoxtide-8T), nor a phosphopeptide whose serine at pThr-1 was mutated to valine (PoloBoxtide-7V; Figure 5B).

Figures 6A, 6C, and 6D are immunoblots showing that Plk-1 PBD interacts with Thr_{130} of mitosis-dependent phosphorylated Cdc25C from HeLa cells. Figure 6A is an anti-CDC25 western blot on lysates from HeLa cells arrested in interphase with aphidicolin or in G2/M with nocodazole, incubated with a GST fusion of the Plk-1 PBD (residues 326-603). Endogenous Cdc25C from mitotic lysates was precipitated with GST-Plk-1 PBD and detected by anti-Cdc25C (Santa Cruz Biotechnology). Interaction of GST-Plk-1 PBD with Cdc25C was disrupted as in Figure 5B by pre-incubation of GST-Plk-1 PBD with its optimal phosphopeptide ligand (PoloBoxtide-optimal) but not with the PoloBoxtides-8T or -7V. Figure 6B is a sequence alignment showing that a consensus motif for the Polo-box Domain of Plk-1 is conserved between human (SEQ ID NO: 80) and *Xenopus* Cdc25C (SEQ ID NO: 81). T130 and T138 of human and *Xenopus* Cdc25C, respectively, are known to be phosphorylated during mitosis (Figure 6B). Lysates were prepared from HeLa cells transfected with either wild type, T130A, or S129V HA-Cdc25C (human), arrested in G2/M with nocodazole, and normalized for equal loading of the mitotically up-shifted form. Interaction of GST-Plk-1 PBD (residues 326-603) with mitotically phosphorylated Cdc25C from these lysates was detected by pull-down with glutathione beads, separation by

11.4% SDS-PAGE and anti-HA blotting (Figure 6C). Figure 6D shows lysates, analyzed by 9% SDS-PAGE to enhance separation of the hyper-phosphorylated (P) form of Cdc25C from partially phosphorylated and unphosphorylated (U) forms.

Figure 7A is a set of micrographs visualized using fluorescence microscopy. Figure 7B is a histogram showing the ratio of centrosomal localization by the GST-PBD relative to centrosomal γ-tubulin. U2OS cells were arrested in G2/M with nocodazole and then incubated with 4 μM GST-Plk-1 PBD (residues 326-603) in cell permeabilization buffer containing 1 U /ml Streptolysin-O in the presence of no peptide (upper panel), 250 μM of the optimal phosphopeptide (optimal, middle panel), or 250 μM of the corresponding unphosphorylated analogue (8T, lower panel). Following incubation, the cells were washed extensively, fixed with paraformaldehyde, extracted with Triton X-100, immunostained for GST and γ-tubulin, and counterstained with DAPI to visualize the nucleus. Overlap of the GST (Alexa Fluor 488) and γ-tubulin (Texas Red) signals is shown in the merged figure in the far right column (Figure 7A). The ratio of centrosomal localization by the GST-PBD relative to centrosomal γ-tubulin levels is shown in Figure 7B.

Figure 8 is a schematic diagram showing a model for 2-step activation of Cdc25 and Cdc2/Cyclin B auto-activation through Plk-1. Phosphorylation of a few molecules of Cdc25, either by a small amount of de-repressed Cdc2/Cyclin B or another proline-directed kinase early in mitosis, primes those Cdc25 molecules for binding of Plk-1 through its PBD. Activation of the Plk-1 kinase domain by Plkk1 generates the first wave of Cdc25 activation, dephosphorylating more Cdc2/Cyclin B, which, in turn, phosphorylates additional Cdc25 molecules for interaction with the Plk-1 PBD. The net result is a positive feedback loop for Cdc2/Cyclin B activation (circled).

Figure 9A is a table showing the conservative mutations at the pT-1 serine that abolish Plk1 PBD / peptide binding in solution. Isothermal titration

calorimetry was used to determine binding affinities. The Plk1 PBD (residues 326-603) was expressed in *E.coli* as a GST fusion, purified on glutathione agarose, proteolytically digested from GST, and further purified by anion exchange chromatography. N.D.B. indicates no detectable binding for a Plk1 PBD concentration of at least 150 μM. pT denotes phosphothreonine. Throughout Figures 9A and 9B, the domains are depicted as follows: kinase: white; PC: gray; PB1: red; PB2: blue;

Figure 9B is a filter array that shows binding of GST-Plk1 PBD (residues 326-603) to peptide spots, comprising single point mutants of the Plk1 PBD optimal phosphopeptide (right column). Bound GST-Plk1 was detected by blotting with HRP-conjugated anti-GST antibody.

Figure 10A is a schematic diagram showing the boundaries of the PBD by limited proteolysis. Domain architecture of full-length Plk1 and stable fragments (left) are shown together with the time-course of V8 protease digestion (right). Molecular weight and amino acid boundaries of the limiting domain were determined by mass spectroscopy.

Figure 10B is a schematic diagram showing the Polo-box 1 and Polo-box 2 $\beta_6\alpha$ structures, colored as in (A), are shown superimposed.

Figure 10C is a RIBBONS representation (Carson, 1991) of the structure of the Plk1 PBD in complex with a phosphothreonine-containing peptide shown as a ball and stick representation in yellow. The Polo-boxes and Polo-cap region are colored as in (A). The phosphopeptide binds at one end of a pocket formed between the two polo boxes.

Figure 11A shows a structure-based sequence alignment of the Polo-box Domain family. The following sequences are shown: HsPlk1 (SEQ ID NO: 82), MmPlk1 (SEQ ID NO: 83), RnPlk1 (SEQ ID NO: 84), CePlk1 (SEQ ID NO: 85), DmPolo (SEQ ID NO: 86), XlPlx1 (SEQ ID NO: 87), HpPlk1 (SEQ ID NO: 88), HsPlk2 (SEQ ID NO: 89), MmPlk2 (SEQ ID NO: 90), RnPlk2 (SEQ ID NO: 91), CePlk2 (SEQ ID NO: 92), XlPlx2 (SEQ ID NO: 93), HsPlk3 (SEQ ID NO: 94),

MmPlk3 (SEQ ID NO: 95), RnPlk3 (SEQ ID NO: 96), XlPlx3 (SEQ ID NO: 97), SpPlo1 (SEQ ID NO: 98), and ScCdc5 (SEQ ID NO: 99). Residues with 100% conservation are shaded purple while highly conserved residues are shaded cyan.

Figure 11B is an image of the molecular surface of the PBD based on the structure determined by X-ray crystallography. The surface positions corresponding to the conserved residues are colored as in Figure 11A. The most highly conserved residues within the Plk1 PBD are located exclusively on the peptide-binding face of the PBD. The most highly conserved residues within the Plk1 PBD are located exclusively on the peptide binding face of the PBD. The coloring scheme is as in 11A.

Figure 11C is a schematic diagram depicting the electrostatic potential of the PBD phosphopeptide pocket, calculated using GRASP (Nicholls et al., 1991), with the phosphopeptide superimposed in stick representation (oxygen atoms, red; nitrogen atoms, blue). Negative potential of the PBD surface is colored red and positive potential blue.

Figure 11D is a schematic representation of the interactions between the phosphopeptide (blue) and the Plk1 PBD. Hydrogen bonds, van der Waals interactions, and water molecules are denoted by dotted lines, purple crescents, and green circles, respectively.

Figure 11E is a schematic representation of direct and indirect hydrogen bonds (dotted lines) between the phosphate and the Plk1 PBD. Hydrogen bond lengths are given in angstroms.

Figure 12A is a schematic diagram showing a comparison of the β-sandwich folds of the Plk1 PBD and the Sak polo-box dimer. Tertiary structures are shown on the top together with secondary structure topology (triangles, β strands; rectangles, α-helices) on the bottom. PB1 and PB2 of Plk1 are denoted by red and purple colors, respectively, while the Pc of Plk1 is shown in green. Polo-boxes from separate Sak molecules within the dimer are likewise denoted by

red and purple. The Sak β sandwich involves strand swapping between separate polo-boxes within the dimer.

Figure 12B is a sequence alignment of the Polo-boxes from Plk1 (HsPlk1_pb1, SEQ ID NO: 100; and HsPlk1_pb2, SEQ ID NO: 101) and Sak (SEQ ID NO: 102). Plk1 has a β6α secondary topology while Sak has a circularly altered β5αβ topology. β-sheet and α-helix notation follows PB1; the corresponding elements for PB2 are β7 through β12 and αC. A conserved salt-bridging interaction initially observed in the Sak structural analysis (Leung et al., *Nat. Struct. Biol.* 9:719-724, 2002) is shown by the blue bracket. Conserved non-polar residues are highlighted in blue and residues conserved between Sak and at least one of the Plk1 PBDs are boxed.

Figure 13A is an autoradiograph. Wild type and mutant Plk1 PBD (residues 326-603) were translated *in vitro* in the presence of ^{35}S-methionine and examined for binding to an immobilized pThr-Pro-oriented library and its unphosphorylated counterpart. pTP= biotin-ZGZGGAXXBXpTPXXXXAKKK SEQ ID NO:~~21~~ 30, TP= biotin-ZGZGGAXXBXTPXXXXAKKK SEQ ID NO:~~22~~ 31, where pT is phosphothreonine, Z is aminohexanoic acid, X is all amino acids except Cys, and B denotes a biased mixture of the amino acids P, L, I, V, F, M, W.

Figure 13B is a diagram showing isothermal titration calorimetry results. A H538A/K540M mutation of the Plk1 PBD abolishes binding to its optimal phosphopeptide as measured by isothermal titration calorimetry.

Figure 13C is a Western blot showing that mutation of the H538/K540 pincer disrupts interaction of the isolated Plk1 PBD with Cdc25 *in vivo*. HeLa cells were transfected with wild type and mutant versions of a His-Xpress-tagged Plk1 PBD construct (residues 326-603) or with a control Plk1PBD construct lacking the second Polo-box (residues 326-506) and arrested in G2/M with nocodazole. The Plk PBD was pulled down with Ni^{2+} beads and bound endogenous proteins analysed by SDS-PAGE and blotted for Cdc25.

Figure 13D is a Western blot showing that mutation of the H538/K540 pincer in the Plk1 PBD disrupts interaction of full-length Plk1 with Cdc25 *in vivo*. HeLa cells were transfected with wild type and mutant versions of full-length myc-tagged Plk1 and arrested in G2/M with nocodazole. Plk-myc was immunoprecipitated with anti-myc-conjugated beads and Cdc25 binding to Plk1 analyzed as in 13C.

Figure 14 is a series of photomicrographs showing that mutation of the H538/K540 pincer sequence abolishes centrosomal localization of the Plk1 PBD in HeLa Cells. U2OS cells were arrested in G2/M with nocodazole and then incubated with 4 μM wild-type or mutant GST-Plk1 PBD (residues 326-603) in cell permeabilization buffer containing 1 U/ml Streptolysin-O. Following incubation, the cells were washed extensively, fixed with paraformaldehyde, extracted with Triton X-100, immunostained for GST and γ-tubulin, and counterstained with DAPI to visualize the nucleus. Overlap of the GST (Alexa Fluor 488) and γ-tubulin (Texas Red) signals is shown in the merged figure in the far right column.

Figure 15 is a series of diagrams showing the results of FACS analysis. HeLa cells were transfected with wild type and mutant GFP-tagged Plk1 (residues 326-603) for 32 hours. Cells were harvested, stained with Hoechst 33342, and analyzed by FACS to determine DNA content in the total cell populations (left panels). Similar analysis limited to the transfected cell population was performed by gating only on the GFP expressing cells (right panels). G2/M population percentages are averages from three independent experiments.

Figure 16A is a Western blot that phosphopeptide binding by full-length Plk1 is reduced relative to that for the isolated Plk1 PBD. Approximately 10% of input full length Plk1 (residues 1-603) interacted with an immobilized pThr-Pro oriented library with slight preference over the unphosphorylated library analogue. The phosphorylation-dependent component of binding arose from the PBD, as it was eliminated by mutation of the His538/K540M pincer. In contrast,

phosphopeptide binding by the isolated PBD (Figure 13A) was 10-fold greater and considerably more phospho-dependent.

Figure 16B is a graph showing that the optimal PBD phosphopeptide stimulates full-length Plk1 kinase activity. GST-Plk1 (prepared in SF9 cells) was preincubated without peptide (closed circles), with 250 μM of the optimal PBD phosphopeptide (open squares) or with 250 μM of the non-phosphorylated optimal peptide counterpart (closed squares) for 5 minutes at room temperature prior to initiating the kinase reaction by addition of ATP. [32P]-incorporation into casein was determined by SDS-PAGE electrophoresis, autoradiography, and densitometry. Pre-incubation with the optimal PBD phosphopeptide ligand enhanced the rate of casein phosphorylation by Plk1 by a factor of 2.6 as determined from three independent experiments.

Figure 16C is a schematic diagram depicting a model for Plk1 regulation by the PBD. PB1 and PB2 are shaded orange, kinase domain cyan, phosphopeptide purple with phosphate in red. Inhibitory interactions between the PBD and the kinase domain in the basal state (left) are relieved by phosphopeptide binding, which may also stabilize association of the two Polo-boxes (right).

Figure 17A is an autoradiograph showing the identification of phosphoSer/Thr-binding domains using an ATM/ATR-motif library. An oriented (pSer/pThr) phosphopeptide library, biased toward the phosphorylation motifs for ATM/ATR kinases, was immobilized on Streptavidin beads. This phosphopeptide library [pSQ= biotin-ZGZGGAXXXB(pS/pT)QJXXXAKKK (SEQ ID NO:~~23~~ 35)] and its non-phosphorylated counterpart were screened against *in vitro* translated ^{35}S-Met labeled proteins. (pS/pT) denotes 50% phosphoserine and 50% phosphothreonine; Z indicates aminohexanoic acid; B represents a biased mixture of the amino acids A, I, L, M, N, P, S, T, V; and J represents a biased mixture of 25% E, 75% X, where X denotes all amino acids except Arg, Cys, His, and Lys. PTIP, denoted by arrow, was isolated from pool EE11 as a clone that associated preferentially with

the phosphorylated form of the immobilized peptide library. In each panel, the first and second lanes show binding of proteins within the pool to the phosphorylated and non-phosphorylated libraries, respectively. Identification of PTIP occurred through progressive subdivision of the EE11 pool to a single clone (panel on right denoted by asterisk). Longer exposures revealed partial translation or proteolytic breakdown products of PTIP that also exhibit phospho-discrimination, suggesting that the full-length transcript likely contains a smaller discrete phospho-binding domain. The uppermost band is a fusion artifact of PTIP with vector sequences resulting from translation initiation at an upstream ATG in the vector.

Figure 17B is an autoradiograph showing deletion mapping of the phospho-binding domain of PTIP. Truncations of PTIP were translated *in vitro* and assayed for selective binding to the phosphorylated peptide library as in Figure 17A. Shaded regions in the C-terminus of PTIP correspond to its BRCT domains. Truncation constructs were designed according to boundaries of sequence homology within the BRCT domain, boundaries from sequence alignments, and from the Pfam-delineated BRCT domains (Bateman *et al.*, *Nucleic Acids Res* 27: 260-2, 1999).

Figure 18A is an autoradiograph. PTIP, BRCA1, MDC1, 53BP1 and Rad9 tandem BRCT domains were translated *in vitro* in the presence of 35S-methionine and tested for binding to immobilized phosphopeptide and non-phosphopeptide libraries as described in Figure 17A. The peptide libraries used were pSQ as defined in Figure 17A. pS= biotin-ZGZGGAXXXXpSXXXXXAKKK SEQ ID NO:~~24~~ 36; pT=biotin-ZGZGGAXXXXpTXXXXXAKKK SEQ ID NO:~~25~~ 32, where pS is phosphoserine, pT is phosphothreonine, Z indicates aminohexanoic acid, and X denotes all amino acids except Cys. Both PTIP and BRCA1 tandem BRCT domains display stronger binding to the pSQ and pS libraries as compared to the non-phospho libraries. Domain boundaries: PTIP as indicated in Figure 1 (SEQ ID NO:~~26~~ 15); BRCT1 and 2: amino acids 1634-1863 of SEQ ID NO:~~27~~ 12;

BRCT1 alone: amino acids 1634-1751 of SEQ ID NO:~~27~~ 12; BRCT2 alone: 1725-1863 of SEQ ID NO:~~27~~ 12; MDC1: amino acids 1880-2089 of SEQ ID NO: ~~28~~ 37(NP_055456.1); 53BP1: amino acids 1700-1972 of SEQ ID NO:~~29~~ 38 (NP_005648.1); Rad9: amino acids 1025-1309 of SEQ ID NO:~~30~~ 39 (NP_010503.1).

Figures 18B and C are autoradiographs showing that the PTIP and BRCA1 BRCT domains show strong selection for Phe at the (pSer/pThr)Gln +3 position (7.0 or 7.5), respectively. Tandem BRCT domains of PTIP and BRCA1 were immobilized as glutathione-S-transferase (GST) fusion proteins on glutathione beads and incubated with non-biotinylated versions of the oriented degenerate phosphopeptide libraries described in Figure 17A (XXXB-pS/pT-QJXXX, SEQ ID NO: 118). Following extensive washing, bound peptides were eluted and sequenced. Bar graphs show the relative abundance of each amino acid at a given cycle of sequencing compared to its abundance in the starting peptide library mixture, as described (Yaffe et al., *Methods Enzymol* 328:157-70, 2000).

Figures 18D,18E, 18F, and 18G show binding of GST-PTIP and BRCA1 tandem BRCT domains to a filter array of peptide spots, comprising single point mutants of the optimal BRCT domain phosphopeptide (left column). Bound GST-BRCT domains were detected by blotting with HRP-conjugated anti-GST antibody. The resulting consensus binding motif is indicated in the right column, SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 121, and SEQ ID NO: 122 for Figures 18D, 18E, 18F, and 18G, respectively; wherein X denotes no dominant selection, ϕ denotes residues with aliphatic or aromatic side chains, and letters enclosed in square brackets are specifically de-selected. The top row indicates the amino acid that was substituted for the optimal amino acid. Substitution of pSer for pThr enhanced binding for both PTIP and BRCA1 BRCT domains, consistent with the ITC results. Substitution of pTyr for pThr eliminated binding altogether, verifying that tandem BRCT domains are pSer/pThr-specific binding modules. Replacement of pThr with Thr, Ser or Tyr abrogated tandem BRCT domain

binding. The pTQ oriented blots on the left show strong selection at several positions for both PTIP and BRCA1 BRCT domains; especially for Phe in the +3 position in agreement with the oriented peptide library screening data. The pS oriented blots on the right show that the +3 position is the most important position for peptide selection.

Figure 19A is a Western blot. Lysates from U2OS cells were obtained prior to and 2 hours after the cells were exposed to 10 Gy of ionizing radiation (IR). The lysates were incubated with GST-PTIP tandem BRCT domains, and bound proteins were detected by blotting with the anti-ATM/ATR phosphoepitope motif antibody. Interaction of the PTIP BRCT domains with these phosphoproteins from IR treated cells was disrupted by pre-incubation with the pSQ peptide library, but not with the SQ peptide library or the pTP library.

Figure 19B is a Western blot showing that the interaction of the PTIP BRCT domains with DNA damage induced phosphoproteins from IR treated U2OS cells was disrupted by pre-treating the cells with caffeine (25 mM) prior to IR exposure or by pre-incubating the beads with an optimal BRCT-binding peptide (BRCTtide-opt), but not by preincubating the beads with the peptide's non-phosphorylated counterpart (BRCTtide-7T).

Figure 19C is a Western blot showing that tandem BRCT domains of PTIP interact with 53BP1 following DNA damage. Endogenous 53BP1 from IR treated U2OS cells was precipitated with GST-PTIP tandem BRCT domains and detected by incubating with an anti-53BP1 antibody. Interaction of GST-PTIP tandem BRCT domains with HA-tagged 53BP1, was then detected by anti-HA blotting. This interaction was abolished by treating the lysates with lambda phosphatase, by pre-incubating the beads with an optimal BRCT-binding peptide (BRCTtide-opt), but not with its non-phosphorylated counterpart (BRCTtide-7T), or by preincubating the beads with the pSQ library, but not by preincubating with the SQ library or the pTP library. Treatment of the cells with 25 mM caffeine also disrupted the interaction.

Figure 19D is a Western blot. Lysates from U2OS cells 2 hours following IR were incubated with GST-BRCA1 tandem BRCT domains. DNA damage-induced phosphoproteins were detected by blotting with the anti-ATM/ATR phosphoepitope motif antibody. The interaction of the GST-BRCA1 tandem BRCT domains with the phosphoproteins were disrupted as in panel B. These results show that tandem PTIP and BRCA1 BRCT domains associate with DNA damage-induced phosphoproteins through their phosphopeptide-binding pockets.

Figures 20A-C are photomicrographs showing immunofluorescence in U2OS cells demonstrating that full length PTIP forms DNA damage induced foci and co-localizes with (pSer/pThr)-Gln proteins, 53BP1, and γ-H2AX. Figure 20A shows U2OS cells transfected with a full length PTIP-GFP construct (PTIP-FL residues 1-757). Figure 20B shows U2OS cells transfected with a PTIP deletion construct in which the last two BRCT domains were removed (PTIP-ΔBRCT, residues 1-550). Figure 20C shows U2OS cells transfected with a PTIP construct containing only the last two BRCT domains $(BRCT)_2$, residues 550-757). In Figures 20A-20C, 24 hours following transfection cells were either treated with 10 Gy of ionizing radiation or mock irradiated, allowed to recover for 2 hours, stained, and analyzed by immunofluorescence microscopy.

Figures 21A and B are photomicrographs showing immunofluorescence in U2OS cells demonstrating that caffeine attenuates recruitment of PTIP to DNA damage foci in response to ionizing radiation. U20S cells transfected with full-length PTIP-GFP cDNA were mock treated or pretreated with 10mM caffeine for 70 minutes before exposure to 10Gy ionizing radiation. (A) In reponse to IR, mock-treated U20S cells formed nuclear foci containing PTIP (in green) and H2AXp (in red); these two proteins co-localize at sites of DNA damage (merge). (B) In response to IR, caffeine treated U20S cells formed reduced numbers of nuclear foci; PTIP was mislocalized and did not form discrete nuclear foci (in green) and there were reduced numbers of H2AXp (in red) containing foci;

pretreatment with caffeine effectively abolished co-localization of PTIP and H2AXp (merge).

Figure 22 shows the PTIP amino acid sequence (SEQ ID NO: 15).

Figure 23 shows the PTIP nucleic acid sequence (SEQ ID NO: 13).

Figure 24 shows the BRCA1 amino acid sequence (SEQ ID NO: 12).

Figure 25 shows the BRCA1 nucleic acid sequence (SEQ ID NO: 11).

Figure 26 shows the MDC1 amino acid sequence (SEQ ID NO: 37).

Figure 27 shows the MDC1 nucleic acid sequence (SEQ ID NO: 40).

Figure 28 shows the 53BP1 amino acid sequence (SEQ ID NO: 38).

Figure 29 shows the 53BP1 nucleic acid sequence (SEQ ID NO: 41).

Figure 30 shows the Rad9 amino acid sequence (SEQ ID NO: 39).

Figure 31 shows the Rad9 nucleic acid sequence (SEQ ID NO: 42).

Description of the Invention

The present invention features a method for identifying kinase targets, an exemplary kinase target, the Polo box domain of the Polo-like kinase, and exemplary peptide mimetics that interfere with signaling by the Polo-like kinase.

We have developed a proteomic approach that allows us to identify virtually any peptide-binding domain by simultaneously screening a polypeptide expression library with a biased peptide library. We have used this method to identify, for example, targets downstream of kinases in signaling pathways. This strategy involves using an immobilized library of partially degenerate phosphopeptides, biased toward a kinase phosphorylation motif, to isolate interacting effector proteins targeted by substrates of that kinase. Using this approach for cyclin-dependent kinases, we identified the Polo-box Domain (PBD) of the mitotic kinase Plk-1 as a phosphoserine/threonine binding domain. Polo-like kinases (Plks) perform crucial functions in cell-cycle progression and multiple stages of mitosis. Plks are characterized by the presence of a C-terminal non-catalytic region containing two tandem Polo-boxes, termed the Polo-box domain (PBD).

In addition, we have discovered that the PBDs of human, *Xenopus,* and yeast Plks all recognize similar phosphoserine/threonine-containing motifs. The 1.9Å X-ray structure of a human Plk1 PBD-phosphopeptide complex shows that the Polo-boxes $\beta_6\alpha$ structures. They associate to form a novel 12-stranded β-sandwich domain, to which the phosphopeptide-binds within a conserved, positively-charged cleft located at the edge of the Polo-box interface. Mutations designed to specifically disrupt phosphodependent interactions abolish cell-cycle dependent localization and provide compelling phenotypic evidence that PBD-phospholigand binding is necessary for proper mitotic progression. In addition, phosphopeptide-binding to the PBD stimulates kinase activity in full-length Plk1, suggesting a conformational switching mechanism for Plk regulation and a dual functionality for the PBD. Together, our data reveal a central role for PBD-

phosphoprotein interactions in many, if not all, cellular functions of Plks. This finding provides a structural explanation for how Plk-1 localizes to specific sites within cells in response to Cdk phosphorylation at those sites.

Activation of signaling cascades in eukaryotic cells involves the directed assembly of protein-protein complexes at specific locations within the cell. This process is controlled by protein phosphorylation on serine, threonine and/or tyrosine residues that directly or indirectly regulate protein-protein interactions, often through the actions of modular binding domains. Historically, studies of phospho-binding domains have focused on SH2 and PTB domains, which bind to specific phosphotyrosine-containing sequence motifs. Until recently, it was thought that phosphorylation of proteins on serine and threonine residues was not responsible for direct interactions with modular binding domains but instead induced conformational changes to regulate function. However, a number of domains (14-3-3 proteins, FHA domains, WD40 repeats of F-box proteins, MH2 domains and the WW domain of the prolyl isomerase Pin1) have been identified that bind directly to short phosphoserine or phosphothreonine-containing sequences to control cell cycle progression, coordinate the response to DNA damage, and regulate apoptosis.

The vast majority of intracellular proteins are phosphorylated on serine or threonine residues at some point during their lifetime. Furthermore, known phosphoserine/threonine binding domains comprise a diverse structural group, demonstrating that many divergent tertiary folds have acquired a phospho-dependent binding function through evolution. Approximately one-third of the modular protein domains identified by Pfam and SMART on the basis of sequence homology have no known function. Our technique enables the identification of additional phosphopeptide binding modules that target serine/threonine residues.

2x2 Biased Library Screening

To design a general proteomic screen capable of identifying novel phosphoserine/threonine binding modules, we took advantage of the observation that protein kinases and phosphopeptide binding domains seem to have co-evolved to recognize overlapping sequence motifs (Yaffe et al., *Nat. Biotechnol.* 19:348-353, 2001; Obata et al., *J. Biol. Chem.* 275:36108-36115, 2000). For example, the basophilic protein kinase, Akt, phosphorylates substrates at sites that contain the core motif RXRSX[S/T] (SEQ ID NO: 43) and 14-3-3 proteins bind to a subset of these phosphorylated sites that have the optimal motif RSX[pS/pT]XP (SEQ ID NO: 44). Cyclin-dependent kinases (Cdks) phosphorylate substrates at [S/T]PXR (SEQ ID NO: 45) motifs, and the WW domain of the proline isomerase Pin1 recognizes the phosphorylated forms of these [pS/pT]P sites to mediate isomerization of the proline residue. Importantly, this apparent overlap between kinase and phospho-binding motifs is not perfect. Instead, limited overlap allows combinatorial interactions between substrates of particular kinases and downstream binding modules.

Our motif-based strategy for identifying pSer/Thr-binding domains involved biasing a library of partially degenerate phosphopeptides towards the phosphorylation motif of a kinase and then using an immobilized form of this library as bait in a screen for interacting proteins translated *in vitro* from a cDNA library.

Using a library of phosphopeptides biased towards motifs phosphorylated by cyclin-dependent kinases (Cdks), we identified the C-terminal Polo-box containing region of the human Polo-like kinase, Plk-1, as a specific phosphopeptide recognition module. It has been previously shown that this non-catalytic region is critical both for Polo kinase subcellular localization and for proper mitotic progression in yeast and human cells. Our findings provide the first description of a biochemical mechanism through which Plk-1 performs these essential mitotic functions. Furthermore, the identification of the conserved Plk-1

PBD as the latest member of the growing superfamily of pSer/Thr-binding domains suggests that phospho-specific docking may be a general mechanism for Ser/Thr kinase signaling in eukaryotic biology.

To identify pSer/Thr-binding domains involved in cell cycle regulation, we designed a pThr-Pro-oriented peptide library biased to resemble the motif that would be generated by the action of cyclin-dependent kinases and MAP kinases, as well as that recognized by the mitotic phosphoprotein-specific monoclonal antibody MPM-2, whose pSer/Thr-binding motif we had determined previously (Yaffe et al., *Science* 278:1957-1960, 1997). The library was constructed with a flexible linker and an N-terminal biotin tag, allowing an immobilized form of this library to be used as bait in an interaction screen against a library of proteins produced by *in vitro* expression cloning (Lustig et. al., *Methods Enzymol* 283:83-99, 1997; Figure 1A).

This library vs. library screening approach is the reverse of a traditional peptide library screen in which a single purified domain is assayed against a degenerate peptide library to reveal the optimal binding motif. In the approach presented here, a degenerate but motif-biased peptide library is used to screen for novel binding domains. By using a collection of peptides biased towards the motif of a protein kinase superfamily, the screen casts a larger net than would be possible if only a single peptide were used as bait. To control for phospho-independent peptide binding, an identical library was constructed with Thr substituted for the fixed pThr residue (Figure 1A).

The pThr-Pro-oriented peptide library, and its non-phosphorylated Thr-Pro library counterpart were immobilized on Streptavidin beads and screened in parallel against 680 individual pools of *in vitro* translated [^{35}S]-labeled proteins. Each pool contains ~30 radiolabeled proteins/pool that are detectable by SDS-PAGE/autoradiography (Figure 1B, "pool" lanes). As shown in Figure 1B, proteins produced by *in vitro* translation often failed to bind either library at all or bound more strongly to the non-phosphorylated peptide library-containing beads.

However, we identified 7 distinct pools containing radiolabeled translation products that bound preferentially to the pThr-Pro library compared with the Thr-Pro library (asterisks in Figure 1B).

Plasmid pools containing these positively scoring hits were progressively subdivided and re-screened for phospho-binding until individual clones were isolated and sequenced. Of the 7 positive clones, 3 were successfully recovered, two of which are reported here. One of the clones, 109-B7, was found to encode the prolyl isomerase Pin1, which is known to bind and isomerize pThr-Pro motifs recognized by the monoclonal antibody MPM-2. Its isolation, therefore, validated the feasibility of our screening approach.

A second positively scoring hit, clone 407-C6, was found to encode the C-terminal 80% of the mitotic kinase Plk-1 (polo-like kinase-1, amino acids 95-603). This clone was missing critical components of the Plk-1 kinase domain, including the glycine rich loop (amino acids 60-66) and the invariant lysine (K82), implying that phosphopeptide binding was independent of Plk-1 kinase activity. Phospho-specific binding by the full-length transcript of this incomplete Plk-1 clone was less pronounced than binding by Pin1 (Figure 1B). Partial translation products or proteolytic breakdown fragments arising from this clone (Figure 1B, arrowheads) showed strong discrimination for the phosphorylated peptide library, suggesting that these fragments included a functional phosphopeptide binding domain.

Identification of Polo-Box Domain as a Phosphopeptide Recognition Module

A hallmark feature of the Polo kinase family is the presence of a highly conserved C-terminal region downstream from a conserved amino-terminal kinase domain (Figures 2A and B). This region includes two blocks of strong homology, termed Polo Boxes. To define the limiting fragment of Plk-1 responsible for phosphospecific binding, we generated a series of deletion constructs based on an alignment of the C-terminal regions of human Plk-1, *Xenopus* Plx-1 and *Drosophila* Polo (Figure 2B), and analyzed these deletion fragments for

phosphopeptide-specific binding. As shown in Figure 2A, a construct that began immediately after the kinase domain and extended to the last residue of the protein (residues 326-603) demonstrated strong and specific binding to the phosphothreonine-proline peptide library compared with the non-phosphorylated control. Notably, this construct was superior to the parent clone 407-C6 in discriminating for phosphopeptides. Neither of the individual Polo Boxes alone (denoted PB1 and PB2), nor a construct containing both Polo Boxes but lacking the linker region between the kinase domain and PB1, was capable of phosphopeptide binding (Figure 2A). Furthermore, a construct that included the linker region and PB1 but not PB2 was also unable to bind phosphopeptides. Thus, it appears that the linker region together with both Polo-boxes functions together as a single phosphopeptide-binding module, and we therefore propose that this segment be called the Polo-box Domain (PBD). Intriguingly, this region encompassing both Polo-boxes has been previously shown to regulate the localization of Plk-1 to centrosomes and kinetochores during prophase and to the midbody during late stages of mitosis. Significantly, neither Polo-box alone was sufficient for this localization function, though mutations within PB1 were sufficient to disrupt it.

The Plk-1 Polo-box Domain Consensus Motif

A central feature of our screen for phosphopeptide-binding domains is that any pSer/Thr-binding domain identified through interaction with phosphopeptide library-immobilized beads is amenable to subsequent determination of its optimal binding motif using a standard "forward" peptide library screening approach. A GST fusion protein of the Plk-1 PBD was therefore expressed in bacteria, immobilized on glutathione beads, and incubated with degenerate phosphopeptide libraries oriented on a fixed pThr-Pro (Figure 3A) or pSer-Pro motif (Figure 3B). Following extensive washing, the PBD-bound peptides were eluted and sequenced, and the amount of each amino acid in every degenerate position was

compared to that present in the starting library mixture to derive amino acid selectivity ratios. Surprisingly, the Plk-1 PBD displayed an extraordinarily strong and novel selection for Ser in the pThr-1 position when the pThr-Pro library was used. Extremely strong selection for Ser was also observed in the -1 position when the PBD was assayed using the fixed pSer-Pro library. Binding of the PBD to a phosphoserine-containing peptide library is noteworthy in itself, since at least one other family of phosphopeptide-binding modules, FHA domains, appear to bind only to phosphothreonine-containing motifs. The relative selection values observed for Ser in either the pThr-1 or pSer-1 position, 5.9 and 8.1 respectively, are among the largest we have observed for any domain whose specificity has been previously determined by peptide library screening.

Since the Plk-1 PBD was isolated in a screen for domains that bind to pThr-Pro motifs, it was important to determine the relative importance of Pro in the pThr+1 position for PBD recognition. To accomplish this, peptide library screens were performed with libraries containing a fixed pThr residue, a fixed pSer residue, fixed Ser-pThr residues, or fixed Ser-pSer residues (Table 1, Figures 3C, and 3D). Little selection was observed for proline in the pThr/pSer+1 position when serine was not fixed in the pThr/pSer-1 position (Table 1). Inclusion of serine at this position in a Ser-pThr oriented library, however, unmasked a moderate selection (1.7) for proline at pThr+1 (Figure 3C and Table 1). Proline selection (1.8) was also uncovered at this position when a Ser-pSer oriented library was used (Figure 3D and Table 1). Notably, synergistic selection between serine and proline was also observed in reverse such that inclusion of a fixed Pro residue in the peptide libraries led to a higher selection for serine (Table 1).

Table 1, below, summarizes the results obtained from phosphopeptide motif selection screening (SEQ ID NO: 103).

Table1. pT and pS Peptide Motif Selection by Plk-1 Polo Box Domain

-3	-2	-1		+1
M (1.3)	A (1.4)	S (5.9)	**pT**	P
Y (1.3)	H (1.4)	A (1.6)		
H (1.3)	M (1.4)			
F (1.2)	T (1.3)			
K (1.2)	F (1.3)			
I (1.4)	A (1.5)	S (3.7)	**pT**	X
K (1.4)	Q (1.3)	A (1.6)		
	T (1.2)	G (1.3)		
M (1.5)	Q (1.5)	**S**	**pT**	P (1.6)
F (1.4)	A (1.5)			M (1.3)
L (1.2)	H (1.5)			
	M (1.4)			
	F (1.3)			
	T (1.2)			
M (1.7)	T (1.9)	S (8.1)	**pS**	**P**
Y (1.5)	H (1.7)			
H (1.4)	M (1.5)			
F (1.3)	F (1.4)			
K (1.2)				
F (1.4)	T (1.9)	S (6.0)	**pS**	X
M (1.3)	H (1.4)			
Y(1.3)	M (1.3)			
	A (1.3)			
M (1.6)	M (1.6)	**S**	**pS**	P (1.8)
F (1.3)	Q (1.5)			M (1.3)
Y (1.3)	H (1.5)			
L (1.2)	A (1.3)			
	T (1.3)			

A GST fusion of the Plk-1 Polo Box Domain was screened for binding to six phosphopeptide libraries, which contained the sequences MAXXXXpTPXXXXAKKK SEQ ID NO:~~31~~ 46, MAXXXXpTXXXXAKKK SEQ ID NO:~~32~~ 47, MAXXXXSpTXXXXAKKK SEQ ID NO:~~33~~ 48, MAXXXpSPXXXAKKK SEQ ID NO:~~34~~ 49, MAXXXXpSXXXXAKKK SEQ

ID NO:~~35~~ 50, and MAXXXXSpTXXXXAKKK SEQ ID NO:~~36~~ 48, where X indicates all amino acids except Cys. Residues showing strong enrichment are underlined. Selection for Pro (1.4) was observed in the –4 position in the X_4SpTX_4 and X_4SpSX_4 screens. Slight selection for aliphatic and aromatic residues was observed in the +2 position in most screens. Little or no selection was observed in the –5, +3, +4, or +5 positions in any of the screens.

These results suggested that the presence of Pro in the pThr/pSer+1 position, while helpful, was not absolutely required for binding. In agreement with this, the Plk-1 PBD bound in a phospho-specific manner to bead-immobilized peptide libraries containing either a fixed pThr-Pro dipeptide or an isolated pThr alone (Figure 3E). In contrast, the other protein isolated in our screen, full-length Pin1, bound only to the pThr-Pro peptide library beads.

To verify the results of oriented peptide library screening, binding of individual phosphopeptides to the Plk-1 PBD was measured by isothermal titration calorimetry (Figure 4A and 4B). The optimal phosphopeptide ligand (PoloBoxtide-optimal), containing the core sequence Met-Gln-Ser-phoshoThr-Pro-Leu (SEQ ID NO: 51) derived from peptide library screening, bound tightly to the Plk-1 PBD with a dissociation constant of 280 nM. Furthermore, it formed a 1:1 protein/peptide complex, indicating that separate phosphopeptides were not interacting simultaneously with each of the two polo boxes within the PBD. Substitution of threonine for phosphothreonine (PoloBoxtide 8T) resulted in complete loss of binding, reiterating the absolute dependence of interaction on the presence of a phosphate group. Substitution of phosphoserine for phosphothreonine within the optimal PBD motif maintained peptide binding to the Plk-1 PBD in agreement with the peptide library screening results, albeit with a seven-fold drop in affinity. In contrast, substitution of phosphotyrosine for phosphothreonine completely abrogated binding, demonstrating conclusively that the Plk-1 PBD is a pThr/pSer-specific binding domain. The extraordinarily strong selection observed for Ser in the pThr/pSer-1 position within the Plk-1 PBD

binding motif was confirmed using a series of mutant peptides. When this Ser was replaced with either of the sterically small amino acids Ala or Gly, with the hydroxyl containing amino acid Thr, or with the homologous amino acid Cys, no peptide binding was detectable. Moderate selection for Pro in the pThr/pSer+1 position was verified by a greater than five-fold increase in K_d when another β-turn forming residue, Asn, was substituted for Pro in this position. Based on the oriented peptide library screening data (Figure 3, Table 1) and these ITC results, we therefore propose that the core consensus motif recognized by the Plk-1 PBD is S-[pT/pS]-(P/X) (SEQ ID NO: 52).

Physiological Substrates of PBD

The monoclonal antibody MPM-2 (Mitotic Phosphoprotein Monoclonal-2), originally raised against mitotic HeLa cell extracts, recognizes a conserved pSer/pThr-Pro epitope present on ~ 50 phosphoproteins that are localized to various mitotic structures. The initial screen from which the Plk-1 PBD was identified used a peptide library that was partially biased to resemble the MPM-2 epitope. A number of important mitotic regulators that are recognized by this antibody, including Cdc25, Wee1, Myt1, Topoisomerase II alpha and inner centromere proteins (INCENP), contain one or more exact matches of the S-[pS/pT]-P PBD-binding motif. We therefore investigated whether the Plk-1 PBD bound to MPM-2 reactive proteins. HeLa cells were treated with aphidocolin to induce a G1/S arrest or with nocodazole to induce a G2/M arrest and cell lysates were analyzed by immunoblotting (Figure 5A). As expected, the number of MPM-2 reactive proteins was greatly enhanced in the mitotically-arrested cells. Many of these MPM-2 reactive mitotic phosphoproteins were specifically bound by the Plk-1 PBD, suggesting that phosphorylation of these proteins by proline-directed mitotic kinases generated a PBD-binding site. Furthermore, the Plk-1 PBD bound to a different and somewhat smaller subset of MPM-2 epitope-containing proteins than those that bound to Pin1 (Figure 5A), which was expected

given that the MPM-2 epitope motif more closely resembles the optimal consensus motif for Pin1 than that of the Plk-1 PBD.

To determine whether the Plk-1 PBD associates with MPM-2 epitopes through its phosphopeptide binding pocket, peptide competition assays were performed. Pre-incubation of the Plk-1 PBD with its optimal phosphopeptide ligand dramatically inhibited the binding of MPM-2 epitopes (Figure 5B, 'opt'). In contrast, the non-phosphorylated analogue ('8T') or a peptide with Val substituted for Ser in the pT-1 position ('7V') had no effect.

One particular MPM-2 antigen that is also known to be phosphorylated and regulated by Plk-1 and its *Xenopus* homologue is the cell-cycle regulated protein phosphatase Cdc25. We therefore investigated whether Cdc25C associated with the Plk-1 PBD in a cell-cycle-regulated and phospho-specific manner. During mitosis, Cdc25C undergoes a dramatic reduction in gel mobility due to extensive phosphorylation at its N-terminus. The Plk-1 PBD was found to interact only with this mitotically up-shifted form of Cdc25C (Figure 6A). Pre-incubation of the Plk-1 PBD with its optimal phosphopeptide ligand, but not with the 8T or 7V mutant peptides, completely prevented this association, demonstrating that it was mediated through the phosphopeptide binding pocket of Plk-1. During mitosis, Cdc25C is known to be phosphorylated on five conserved Ser/Thr-Pro sites within its N-terminus. One of these sites, Thr_{130} (corresponding to Thr_{138} in *Xenopus* Cdc25C) contains a conserved Plk-1 PBD consensus motif (Figure 6B). To investigate whether this site was important for the Cdc25C-Plk-1 interaction, HeLa cells were transfected with HA-tagged wild-type Cdc25C, or with Thr_{130}Ala or Ser_{129}Val point mutants of Cdc25C expected to disrupt the PBD-binding motif. Following mitotic arrest with nocodazole, the Plk-1 PBD bound strongly only to the wild-type protein, but only very weakly to either of the point mutants, indicating direct interaction between the Plk-1 PBD phosphopeptide-binding pocket and a mitotically-phosphorylated PBD consensus motif in Cdc25C (Figure 6C). Furthermore, both of these point mutants had a decreased electrophoresis

mobility shift when analyzed on lower percentage gels (Figure 6D), suggesting that mutations which impair Plk-1 PBD binding result in incomplete Cdc25C phosphorylation *in vivo*.

Centrosomal localization of the Plk-1 PBD occurs through its phosphopeptide-binding pocket.

Plk-1 localizes to centrosomes and kinetochores in prophase and to the spindle mudstone during late stages of mitosis. Centrosomal localization has been shown to require both the PB1 and PB2 regions, but not kinase activity, since localization is maintained when Lys_{82}, which is mediates phosphate transfer, is mutated to Met. To investigate whether the phosphopeptide binding function of the Plk-1 PBD was critical for its centrosomal localization, U2OS cells were mitotically arrested with nocodazole, permeablized with Streptolysin-O, and incubated with GST-Plk-1 PBD in the absence or presence of peptide competitors. The Plk-1 PBD was observed to localize to the centrosomes of late prophase-arrested cells (Figure 7A), as verified by co-staining with an anti-γ-tubulin antibody.

This centrosomal localization was significantly disrupted in the presence of an optimal Plk-1 PBD phosphopeptide but was unaffected when the assay was performed using the same concentration of the non-phosphorylated peptide analogue (Figures 7A and 7B). This observation, together with published data showing that the C-terminus of Polo-like kinases is essential for their function *in vivo*, strongly suggests that intracellular targeting of Plk-1 to critical substrates is mediated through interaction of the PBD phosphopeptide pocket with phosphorylated motifs in mitotic structures.

The Plk-1 PBD and regulation of mitotic progression by cyclin-dependent kinase priming

Our identification of the Plk-1 PBD as a novel phosphoserine/threonine-binding domain adds another member to the growing superfamily of pSer/Thr-binding modules and demonstrates the general utility of our phospho-motif-based affinity screen for discovering and functionally characterizing novel signaling domains that function downstream of protein kinases. This screening technique can be used to identify binding modules interacting with substrates of any kinase whose phosphorylation motif is known. Other techniques that identify protein-protein and protein-peptide interactions, such as yeast 2-hybrid and phage display approaches cannot be used in screens for phospho-binding domains since reliable and constitutive phosphorylation of a diverse collection of bait sequences is required. A further strength of our technique is that any domain isolated through screening with bead-immobilized peptide libraries yields an optimal consensus binding motif when the domain is subsequently analyzed by traditional peptide library screening. This allows the motif for the pSer/Thr-binding domain to be combined with that of the potential phosphorylating kinase(s) in database searching and protein sequence analysis and should facilitate the proteome-wide prediction of ligands within a common signaling pathway.

The C-terminal region of Polo-like kinases has long been recognized as essential for their *in vivo* function in mitosis and cytokinesis, but its structural mechanism has remained mysterious. Mutations within this region of Plk-1 and its *S. cereviseae* homologue, Cdc5, abolish their ability to rescue a temperature-sensitive mutant of *cdc5* despite the presence of a fully functional kinase domain. When expressed alone, the C-terminal domain of Polo-like kinases localizes to centrosomes and the spindle midzone similar to the full-length kinase, and its overexpression causes mitotic and cytokinetic arrest.

We have shown that the C-terminal domain of Plk-1 is a phosphoserine/threonine-binding module whose phospho-binding pocket binds to

known Polo substrates and mediates localization to subcellular sites where endogenous Polo kinases are found. In the basal state the PBD binds to the kinase domain, inhibiting its phosphotransferase activity. In addition to overcoming this inhibition, maximal activation of the kinase domain also requires phosphorylation in its activation loop by upstream kinases such as xPlkk1/SLK. This requirement for both priming phosphorylation of substrates and activation loop phosphorylation provides a molecular switch that regulates Plk-1 kinase function at discrete stages of the cell cycle. In addition, it provides a potential means for mitotic checkpoint control, since neither phosphorylation of the activation loop nor substrate priming phosphorylation alone would be sufficient for proper activation of Polo kinases *in vivo*.

A number of striking parallels between the PBD of Plk-1, SH2 domains in Src family kinases, and FHA domains in the Rad53/Chk2 family of checkpoint kinases are apparent. Like the Plk-1 PBD, SH2 domains of Src-family kinases both inhibit kinase activity in the inactive state and facilitate substrate targeting when Src kinases have been activated by phosphorylation on their activation loops. In Src kinases, the mechanism of inhibition involves intramolecular binding of the SH2 domain to a pTyr motif at the end of the kinase domain. It remains unknown whether Polo kinase family inhibition by the PBD involves a similar interaction with internal pSer/pThr sites, or whether an alternative PBD surface is involved. Members of the Chk2 kinase family contain one or more pThr-binding FHA domains in addition to the kinase module. The FHA domain(s) are critical for proper Chk2 function in response to DNA damage and for the phospho-dependent targeting of Chk2 into larger multimolecular complexes where activation occurs.

We found the optimal motif for Plk-1 PBD binding to be S-[pS/pT]-P/X. Differences in PBD selectivity for amino acids flanking the pSer/Thr position are likely to be biologically important for the interaction of Polo kinases with their substrates *in vivo*. The primary role of the +1 Pro may be to link phospho-

dependent PBD binding to activation of cyclin-dependent kinases that phosphorylate the motif, providing a means to temporally and spatially regulate the action of Polo-like kinases during mitosis. The absolute requirement for Ser in the –1 position provides strong discrimination for Plk-1 binding to only a limited subset of mitotic kinase substrates. In addition, we found that the motif recognized by the Plk-1 PBD partially overlaps with the proline-directed sequence motif recognized by the monoclonal antibody MPM-2 which reacts against a large number of mitotically phosphorylated proteins, and we demonstrated a direct interaction between the PBD phosphobinding pocket and MPM-2 reactive proteins in pull-down experiments with mitotic cell extracts. This finding provides an elegant explanation for the progressive accumulation of MPM-2 immuno-reactivity and Polo kinase localization observed at maturing centrosomes, and suggests that generation of MPM-2 epitopes by Cdks and other mitotic kinases triggers PBD-mediated recruitment of Polo kinases to specific mitotic structures.

Both Cdks and Polo kinases have been implicated in activating the phosphatase Cdc25, leading to desphosphorylation and activation of Cdc2/Cyclin B and progression through mitosis. The relative roles of Cdks and Polo kinases in Cdc25 activation, however, remains controversial. Our finding that the Plk-1 PBD binds to one or more critical Cdk sites on Cdc25C suggests a molecular rationale for 2-step activation of Cdc25 that has been postulated to drive auto-amplification of Cdc2/CyclinB activity. In prophase, low levels of Cdc2/CyclinB activity are insufficient to fully activate Cdc25, but provide priming phosphorylation of Cdc25 for interaction with the PBD. Subsequent activation of Polo kinases later in mitosis by activation loop kinases such as Plkk1/SLK leads to an initial wave of Cdc25 activation, which generates more Cdc2/Cyclin B activity, primes additional Cdc25 molecules for activation by Polo-like kinases, and results in a positive feedback loop for the production of additional Cdc2/Cyclin B activity (Figure 8). This model is able to explain the result of Toyoshima-Morimoto *et al.* (*EMBO Rep.*, 3:341-348, 2002) that maximal intracellular targeting and activation of

Cdc25, even in the presence of constitutively active Plk-1, still requires the co-expression of Cyclin B1.

Increased levels of Plk expression have been detected in a variety of human tumors and tumor cell lines, and high levels of expression correlate with poor prognosis. The PBD would be an attractive target for the design of anti-proliferative chemotherapeutics since its compact tripeptide binding motif may be particularly amenable to the design of small molecule peptidomimetics.

Optimal phosphopeptide-binding motifs for the PBDs from all members of the human Plk family, *Xenopus* Plx1 and *Saccharomyces cerevesiae* Cdc5p were determined by oriented peptide library screening as described above. Since we initially isolated the Plk1 PBD in a search for domains that recognize a pThr-Pro-containing motif, primary screens were performed using peptide libraries containing a fixed pThr-Pro core flanked on both sides by four degenerate positions. As seen in Tables 2 and 3, the five PBD's examined each selected for distinct but largely overlapping motifs: Plk1 (SEQ ID NO: 104), Plk2 (SEQ ID NO: 105), Plk3 (SEQ ID NO: 106), Plx1 (SEQ ID NO: 107), and Cdc5 (SEQ ID NO: 108).

Table 2 Phosphothreonine Peptide Motif Selection by Human Polo Kinase Family PBDs

-5	-4	-3	-2	-1		+1	+2
Plk1							
	M (1.5) F (1.1)	M (1.3) Y (1.3) H (1.3) F (1.2) K (1.2)	A (1.4) H (1.4) M (1.4) T (1.3) F (1.3)	S (5.9) A (1.6)	**pT**	**P**	F (1.2) I (1.2) K (1.2)
P (1.4) F (1.1)	P (1.5) F (1.3) M (1.3) L (1.2) I (1.1)	M (1.5) F (1.4) L (1.2)	Q (1.5) A (1.5) H (1.5) M (1.4) F (1.3) T (1.2)	**S**	**pT**	P (1.6) M (1.3)	L (1.2) K (1.1) V (1.1)
Plk2							
	F (1.9) I (1.6) M (1.5) L (1.4) P (1.1)	Q (1.9) M (1.8) H (1.6) F (1.3)	T (2.1) H (2.1) Q (1.2)	S (7.5)	**pT**	**P**	F (1.5) L (1.5) I (1.3) V (1.1)
P (2.4) F (1.4) I (1.2)	M (1.5) F (1.5) P (1.4) L (1.4) I (1.3) V (1.2)	Q(1.9) T (1.6) M (1.6) H (1.6) F (1.2)	T (2.8) H (2.0) Q (1.7)	**S**	**pT**	P (1.7)	K (1.5) L (1.2) I (1.1)
Plk3							
	I (1.5) L (1.4) V (1.3) F (1.2) P (1.2)	M (1.6) L (1.3) F (1.3)	T (1.6) H (1.4)	S (3.0)	**pT**	**P**	K (1.3) V (1.2) F (1.2)
P (1.2)	L (1.2) I (1.2)	A (1.5) M (1.2) F (1.2) I (1.2)	T (2.6) H (1.6)	**S**	**pT**	P (1.6) D (1.4) E (1.3)	K (1.4)

GST fusions of the Polo-box Domains (PDBs) from hPlk1, hPlk2, and hPlk3 were screened for binding to phosphopeptide libraries containing the sequences MAXXXXpTPXXXXAKKK (SEQ ID NO: 46) and MAXXXXSpTXXXXAKKK (SEQ ID NO: 48), where X indicates all amino acids except Cys. Residues showing strong enrichment are underlined.

Table 3 Phosphothreonine Peptide Motif Selection by Polo Kinase PBD Orthologs

-5	-4	-3	-2	-1		+1	+2
Plx1							
	F (2.1) I (1.6) L (1.3) M (1.2)	F (1.6) L (1.5) M (1.5)	T (2.1) H (1.7)	S (7.3)	pT	P	I (1.6) L (1.5) V (1.1)
P (1.8) F (1.4)	P (1.6) F (1.5) L (1.5) I (1.4) M (1.3)	F (1.6) M (1.5) L (1.4)	T (3.0) H (1.8) Q (1.3)	S	pT	P (1.9)	K (1.4) I (1.3) L (1.2)
Cdc5							
	M (1.9) L (1.5) I (1.4) F (1.2)	A (2.5) M (1.5) F (1.1)	T (2.4) A (1.8) Q (1.5) M (1.4) H (1.4)	S (5.3)	pT	P	X
P (2.8) F (1.3)	L (2.2) M (1.7) I (1.5) F (1.5) V (1.1)	A (3.4) V (1.3) I (1.2)	A (2.1) Q (1.7) T (1.6) H (1.6) M (1.3)	S	pT	P (1.4)	L (1.3) I (1.1)

GST fusions of the Polo-box Domains (PDBs) from *Xenopus* Plx1 and *S. Cerevisiae* Cdc5p were screened for binding to phosphopeptide libraries containing the sequences MAXXXXpTPXXXXAKKK (SEQ ID NO: 46) and MAXXXXSpTXXXXAKKK (SEQ ID NO: 48), where X indicates all amino acids except Cys. Residues showing strong enrichment are underlined.

All of the PBDs showed unequivocal selection for Ser in the pThr-1 position with selectivity ratios (i.e. the mol% of Ser in the PBD-bound peptides at the pThr-1 position divided by the mol% of Ser in the starting library mixture at the pThr-1 position) ranging from 3.0 to 7.5. Motif similarity occurs even though these PBDs vary considerably in amino-acid sequence and the respective human Plks perform divergent cellular functions. The PBDs as a group consistently demonstrated moderate selection for Thr, His, Gln, and Met in the pThr-2 position. There was general selection amongst all PBDs for aliphatic and aromatic residues in the pThr-3, pThr-4 and pThr+2 positions, although Cdc5p showed a particularly strong and unique selection for Ala in the pThr-3 position, while Plk2 showed strong and unique selection for Gln at this position. All PBDs except Cdc5p also selected for Pro in the pThr-4 position and Lys in the pThr+2 position

Based on these data, secondary peptide libraries containing a fixed Ser-pThr core were used to further refine the motifs and investigate the relative importance of Pro in the pThr+1 position. These screens revealed modest selection for Pro at pThr+1 for all PBDs, with selectivity ratios ranging from 1.4 to 1.9 (Tables 2 and 3). Selection at other motif positions for each PBD was consistent with those obtained using the pThr-Pro library, though we were now able to observe significant and conserved selection for Pro and Phe in the pThr-5 position. (pT-5 was degenerate in the Ser-pThr library, but was a fixed Ala residue in the pThr-Pro-oriented library.) Thus, it appears that the PBDs of all Plks investigated, including all conventional human Plk homologues, select a similar motif that can be most generally represented by the consensus sequence: [Pro/Phe]-[ϕ/Pro]-[ϕ/Ala_{Cdc5p}/Gln_{Plk2}]-[Thr/Gln/His/Met]-Ser-[pThr/pSer]-[Pro/X] SEQ ID NO:~~38~~ 2, where ϕ represents hydrophobic amino acids.

The striking selection observed for Ser in the pThr-1 position in all PBDs was examined in detail for the human Plk1 PBD, which binds to its optimal motif, Pro-Met-Gln-Ser-pThr-Pro-Leu (SEQ ID NO:~~39~~ 6) (Table 2), with a K_d of 280 nM (Figure 9A).

A variety of small side-chain amino-acids were therefore substituted in the pThr-1 position, and peptide binding to the Plk1 PBD measured using isothermal titration calorimetry (ITC) (Figure 9A). Surprisingly, replacement of Ser with Gly, Ala, the hydroxyl-containing amino-acid Thr, or the Ser isostere Cys, completely abrogated Plk1 PBD-phosphopeptide binding. We had previously observed that replacement of Ser at the pThr-1 position with Val, the amino-acid showing the lowest selection in this position, was sufficient to eliminate peptide binding (Elia et al., *Science* 299:1228-1231, 2003). Nevertheless, the finding that replacement of Ser with a variety of chemically similar amino acids also completely disrupted the interaction between the PBD and free phosphopeptides in solution was unexpected.

To extend this analysis, each amino acid in the eight positions flanking the phosphothreonine within the optimal Plk1 PBD binding motif was substituted with each of the remaining nineteen naturally occurring amino acids using a solid phase array of immobilized phosphopeptides (Figure 9B). This conclusively demonstrated that only Ser was tolerated in the pThr-1 position (Figure 9B). Selectivities at other positions were generally consistent with the results of oriented peptide library screening. Cys and Gly, however, were selected at the pThr+1 position at least as strongly as Pro in the immobilized phosphopeptide assay. Cys is routinely omitted during construction of oriented peptide libraries to minimize cross-linking and oxidation effects. Higher relative selection for Gly in the context of immobilized peptides than in solution phase peptide library assays may be due, in part, to the greater entropic penalties associated with ordering Gly residues compared with Pro residues when both ends of a peptide are free. Alternatively, these subtle differences may reflect the fact that the peptide filter assay examines individual point mutations in the context of a single amino-acid sequence, while oriented peptide library screening samples an entire ensemble of sequence motifs simultaneously. Regardless, Pro probably represents the most 'physiological' amino acid in the pThr+1 position, since the phosphorylation event necessary for PBD binding is likely to be catalyzed primarily by Pro-directed kinases such as Cdks and MAP kinases.

Overall Structure of the Plk1 PBD

The boundaries of the minimal PBD within the C-terminal regions of both Plk1 and Cdc5p were determined using limited proteolysis and mass-spectrometry. Studies using V8 protease (Figure 10A) and trypsin (data not shown) indicated that only the last 45 residues of the linker between the kinase domain and the first Polo-box were structured as part of the PBD (Figure 10A). Similar results were obtained using the C-terminal segment of Cdc5p (data not shown). We refer to the beginning of this additional region as the Polo-cap (Pc). For both Plk1 and Cdc5p,

we found no significant difference in the phosphopeptide-binding affinities of fragments encompassing the entire C-terminal regions or the proteolytically-defined PBDs, indicating that the first ~ 40 amino acids between the kinase and the Pc plays no major role in peptide binding. Shorter fragments of both Plk1 and Cdc5p encompassing just the Polo boxes, but lacking the Pc, were insoluble in *E. coli*, indicating a clear structural role for the Pc in both proteins, despite the absence of any extensive sequence homology between the two proteins in this region.

The X-ray structure of a recombinant form of the proteolytically-defined Plk1 PBD (residues 367-603) in complex with its 'optimal' phosphopeptide was solved by multiwavelength anomalous diffraction (MAD) using Se-Met-containing protein, and refined against native data extending to 1.9Å resolution (Table 4).

Table 4 Crystallographic analysis

Data Collection				
Dataset (λÅ)	Native (0.98)	Se (0.97838)	Se (0.97887)	Se (0.95)
	14.1 - SRS		14.2 - SRS	
d (Å)	20.0-1.9	20.0-3.5	20.0-3.5	20.0-3.5
Completeness (%)	97.7	99.9	99.0	99.2
Redundancy[1]	3.6	3.7[3]	~1.9[3]	~1.9[3]
R_{sym} (%)[2]	5.3	5.4[3]	5.2[3]	4.9[3]

Phasing analysis								
Resol bin (Å)	20-11.2	11.2-7.5	7.5-6.0	6.0-5.2	5.2-4.6	4.6-4.2	4.2-3.9	3.9-3.6
FOM	0.79	0.83	0.79	0.70	0.59	0.53	0.48	0.44
Mean FOM	0.60							

Refinement			
R_{cryst} (%)[4]	R_{free} (%)[5]	rms_{bond} (Å)	rms_{angle} (deg.)
24.0	26.8	0.007	1.2

[1] N_{obs}/N_{unique}
[2] $R_{sym} = S_j|<I> - I_j|/S<I>$ where I_j is the intensity of the jth reflection and <I> is the average intensity.
[3] Calculated with Bijvoets separated
[4] $R_{cryst} = S_{hkl} ||F_{obs}| - |F_{calc}|| / S_{hkl} |F_{obs}|$
[5] R_{free} - as for R_{cryst} but calculated on 5% of the data excluded from the refinement calculation.

The structure (Figure 10B) shows that the PBD contains two $\beta_6\alpha$ motifs that comprise the two Polo-box regions (PB1 & 2) identified by sequence profiling. The atomic structural coordinates of this structure are provided in Table 5. In spite of the fact that the amino-acid sequences of the two Polo-boxes within any one Plk exhibit only ~20-25% sequence identity, the structures of the two motifs are quite similar (root mean square (rms) deviation of 77 Cα atoms of 1.6Å; Figure 10B). The two Polo-boxes pack together to form a 12-stranded β-sandwich flanked by three α-helical segments (Figure 10C). Although motifs resembling the Polo-box structure are represented in the Protein Databank, the overall domain structure represents a new protein fold.

The Pc consists of an α-helical segment αA, loop, and short 3_{10} helix which connects to the N-terminal β-strand of Polo-box 1 (β1) through a ~10 residue linker region (L1). The Pc wraps around Polo-box 2 like a hook tethering it to Polo-box 1. αA packs against αC from PB2 in an anti-parallel coiled-coil arrangement, while the 3_{10} helix packs against the shorter αC'. The two Polo-boxes are connected by a second ~30 residue linker sequence (L2) that is partially conserved. L1 and L2 run in anti-parallel directions between the two Polo-box β-sheets. Thus, the hydrophobic core is formed from direct interactions of highly conserved non-polar residues predominantly located on β1/β2 from PB1 and β6/β7 from PB2, together with an array of interactions with the intercalating linker regions.

Novel PBD-Phosphopeptide Interactions are Crucial for Specificity

The phosphopeptide binds in a largely extended conformation to a region of positive charge, located at one end of a shallow cleft formed between the two Polo-boxes (Figure 10). In all, ~1000Å^2 of solvent accessible surface are buried by binding of the seven phosphopeptide residues that are visible in our electron density maps. Binding involves part of an extensive, highly conserved surface that

is located exclusively on the peptide-binding face of the PBD (Figure 11A, 11B). This conserved surface coincides with the only significant region of positive electrostatic potential within the entire PBD (Figure 11C). Overall, the phosphopeptide interacts predominantly with β1 from PB1, the N-terminal end of L2 and β8 and 9 from PB2. Hydrogen bonding interactions formed with the peptide side- and main-chain atoms alternate to some degree between residues within the two Polo-boxes, forming a zipper-like structure at the edge of the PB1/PB2 interface (Figure 11D).

PBD binding to the phosphate moiety involves a combination of direct contacts with protein side-chains together with extensive indirect interactions through a well-defined lattice of water molecules, many of which are fully hydrogen-bonded (Figure 11E). In total, the phosphate group participates in eight hydrogen-bonding interactions explaining the critical dependence on peptide phosphorylation for binding (Elia et al., *Science* 299:1228-1231, 2003). The only residues that contact the phosphate group directly are His-538 and Lys-540 from PB2, whose side chains form a pincer-like arrangement that chelates the O1, O3, and Oγ phosphate oxygens.

The structural basis for the extraordinarily high selectivity for serine at the pThr–1 position results from a major difference in orientation of the bound phosphopeptide when compared with phosphopeptide complexes of 14-3-3 proteins and FHA domains, the two major classes of pSer/pThr binding proteins (Durocher et al., *Mol. Cell.* 6:1169-82, 2000; Yaffe et *al.*, *Cell* 91:961-971, 1997). In these structures, the pThr–1 side-chain is solvent exposed and little selection is observed at this position. In contrast, the peptide orientation in the Plk1 complex is inverted such that the Ser –1 side-chain is directed towards the Plk1 surface (Figure 11B). In this orientation, it engages in two hydrogen bonding interactions with Trp-414 main-chain atoms, and one with the Leu-491 main-chain carbonyl via a water molecule (Figure 11C). Significantly, the Ser –1 Cβ atom makes favourable van der Waals interactions with Cδ1 from the Trp-414 indole side-

chain. This explains why even a conservative replacement of Ser with Thr at this position abrogates peptide binding (Figure 9A), presumably due to a steric clash of the threonine γ-methyl substituent with Trp-414.

The critical role of Trp-414 in ligand binding revealed by our crystal structure (Figure 11D) explains the observation that a W414F mutation eliminates both centrosomal localization of Plk1 and its ability to complement the *cdc5-1 ts* mutation (Lee et al., *Proc. Natl. Acad. Sci. USA* 95:9301-9306, 1998). Both of these effects are likely to be at least partly attributable to disruption of critical Ser-1 interactions with the PBD. In agreement with this, a mutant PBD containing the W414F substitution is severely compromised in phosphopeptide binding, with an affinity of >100 μM as determined by ITC. Loss of binding is unlikely to result from gross structural perturbation of the Polo-box fold, since the mutant PBD exhibits similar secondary structural content to the wild-type protein as judged from far UV CD spectra (data not shown). Furthermore, Trp-414 in Polo-box 1 is replaced by tyrosine in PB2 of both wild-type *S. pombe* Plo1 and *S. cerevisiae* Cdc5p PBD's, (Figure 11A), showing that similar substitutions are naturally tolerated in a related structural context.

Consistent with the oriented library selection, the protein-peptide interface is dominated by interactions of the PBD with the pThr and Ser-1 (Figure 11C, 11D). Although we observed modest selection for Pro at the pThr+1 position, it appears from the structure that it does not contribute greatly to the binding interface, and multiple substitutions at this position are tolerated for peptide binding (Figure 9B). In the PBD structure, the *trans*-proline introduces a kink after the Ser-pThr directing the peptide backbone back toward the binding surface, allowing the pThr+2 main chain amino group to contact the PBD. Thus, the +1 Pro likely increases binding affinity by diminishing the entropic penalty for making this favorable backbone contact. This contrasts with structures of pSer-Pro peptide complexes of both the Pin1 WW and the Cdc4 WD40 domains in which the Pro+1 side chain inserts into a hydrophobic pocket and makes coplanar

interactions with a buried tryptophan (Leung et al., *Nat. Struct. Biol.* 9:719-724, 2002; Verdecia et al., *Nat Struct Biol* 7:639-643, 2000).

Plk1 and Sak Polo-boxes are Structurally Distinct – One Motif, Two Folds

The human Plk family encompasses the canonical kinases (Plks 1-3) and Sak, which contains a highly homologous Ser/Thr kinase domain but only a single divergent Polo-box. Recent structural data has shown that the isolated Polo-box from murine Sak forms an intermolecular dimer, leading to the suggestion that tandem Polo-boxes in Plk1-related Plks may form a related, intra-molecular 'dimeric' architecture (Leung et al., *Nat. Struct. Biol.* 9:719-724, 2002). Our structure shows that this notion is broadly correct. In each case, the Polo-box repeat comprises a six-stranded β-sheet and α-helix. This structural unit associates with a second Polo-repeat via intra- or intermolecular interactions in Plk1 and Sak respectively, to form β-sandwich domain structures. However, closer examination reveals profound differences between the organizations of the two structures (Figure 12A and 12B). The $\beta_6\alpha$ topology of the Plk1 Polo-box is replaced by a circularly-permuted $\beta_5\alpha\beta$ topology in Sak. Consequently, Plk1 β1 has no equivalent in the Sak Polo-box sequence, and instead overlaps structurally with Sak β6. In addition, the Sak β-sheet is completed by a 'segment-swap' of β4 & 5 between monomers. Most strikingly, the association of the two Polo-boxes differs completely such that residues forming the interface between Polo-repeats in the Sak homodimer are located largely on the exterior of the Plk1 β-sandwich, where they partially form the interface with the flanking α-helical segments.

Mutation of the His-Lys Pincer Abolishes Phosphopeptide Binding *in vitro*, Cdc25 Binding *in vivo*, and Centrosomal Localization of the Plk1 PBD

To verify that the key phosphothreonine-interacting residues identified in the X-ray crystal structure were indeed responsible for mediating phospho-

dependent interactions *in vitro* and *in vivo*, we mutated His-538 and Lys-540 of the pThr pincer motif, to either Ala and Met, or Glu and Met, respectively. These mutations severely disrupt phosphopeptide binding in solution as judged by the reduced binding of *in vitro* translated Plk1 PBD to a bead-immobilized pThr-Pro oriented library (Figure 13A) and by ITC (Figure 13B).

During mitotic entry, Cdc2/Cyclin-B and Plk1 cooperate to activate the dual specificity phosphatase Cdc25 through extensive phosphorylation of its N-terminus as part of an amplification loop for Cdc2/Cyclin-B activation (Abrieu et al., *J. Cell. Sci.* 111:1751-1757, 1998; Hoffmann et al., *EMBO J.* 12:53-63, 1993; Izumi et al., *Mol. Biol. Cell* 4:1337-1350, 1993; Izumi et al., *Mol. Biol. Cell* 6:215-226, 1995; Kumagai et al., *Cell* 70:139-151, 1992; Kumagai et al., *Science* 273:1377-1380, 1996; Qian et al., *Mol. Cell. Biol.* 19:8625-8632, 1999; Qian et al., *Mol. Biol. Cell* 12:1791-1799, 2001). Mitotically phosphorylated Cdc25C exhibits a large mobility shift on SDS-PAGE (Kumagai et al., *Cell* 70:139-151, 1992). Cdc25C is phosphorylated on at least five Ser/Thr-Pro sites by Cdc2/Cyclin-B *in vitro* (Izumi et al., *Mol. Biol. Cell* 4:1337-1350, 1993; Strausfeld et al., *J. Biol. Chem.* 269:5989-6000, 1994). One of these sites, Thr-130, occurs within a near-optimal PBD binding motif, Leu-Leu-Cys-Ser-pThr-Pro-Asn (SEQ ID NO: 53). We previously observed that a GST-fusion of the isolated PBD could pull-down wild-type Cdc25C, but not a T130A or S129V Cdc25C mutant, from mitotically-arrested HeLa cell lysates. These data strongly suggested that Cdk priming of Thr-130 generates a binding site for the Plk1 PBD to facilitate full activation of Cdc25C by subsequent Plk1-mediated phosphorylation (Elia et al., *Science* 299:1228-1231, 2003). As shown in Figure 13C, expression of His-Xpress-tagged wild-type Plk1 PBD *in vivo* results in a strong interaction with the mitotically phosphorylated form of endogenous Cdc25C in nocodazole-arrested HeLa cells. However, expression of the His-538/Lys-540 pincer mutants eliminates Cdc25C binding as also observed in cells transfected with a PBD construct lacking the second Polo-box.

To investigate whether the PBD plays a similar substrate-targeting role *in the context of full-length Plk1*, HeLa cells were transfected with myc-tagged wild-type or mutant constructs of full-length Plk1, and interactions between Plk1 and endogenous Cdc25C examined in nocodazole-arrested cells using immunoprecipitation and Western blotting (Figure 13D). We observed a strong *in vivo* interaction between the mitotically upshifted form of endogenous Cdc25C with full-length Plk1 in arrested cells that, somewhat surprisingly, was not increased when a kinase-dead Plk1 mutant (K82R) or a double mutant incorporating a T210D mutation in the T-loop to further expose the kinase-binding cleft were employed as substrate traps. Conversely, mutation of the His-538/Lys-540 phosphate pincer mechanism in full-length Plk1 completely disrupted the *in vivo* interaction between Plk1 and Cdc25C demonstrating that the interaction of full-length Plk1 with full-length Cdc25 in G2/M-arrested cells is mediated primarily through the PBD, rather than its associated the kinase domain. This result is important since it directly demonstrates a requirement for PBD phosphopeptide-binding in substrate targeting in the context of the full-length Plk1 molecule.

Finally, we observed that mutation of the His-538/Lys-540 pincer eliminates targeting of the Plk1 PBD to centrosomes in permeabilized prophase-arrested cells (Figure 6). This finding suggests that the localization of Plk1 to centrosomes observed *in vivo* (Jang et al., *Proc. Natl. Acad. Sci. USA* 99:1984-1989, 2002; Lee et al., *Proc. Natl. Acad. Sci. USA* 95:901-9306, 1998) results from direct interactions between the PBD and phosphorylated centrosomal components. In summary, the results in Figures 13 and 14 show conclusively that the structurally defined His-538/Lys-540 pincer mechanism that is responsible for mediating phosphopeptide binding *in vitro*, plays a similar critical role in substrate targeting *in vivo*.

Phosphodependent Substrate Recognition is Necessary for the Disruption of Mitotic Progression by the Isolated Plk1 PBD

Since the PBD is necessary for targeting Plk1 to primed substrates, its overexpression might be expected to act in a dominant-negative fashion to inhibit correct localization of endogenous Plk1 and, therefore, disrupt Plk1 function *in vivo*. Indeed, overexpression of the C-terminus of Plk1 has been shown to cause mitotic arrest and induce formation of randomly oriented, disorganized spindles (Jang et al., *Proc. Natl. Acad. Sci. USA* 99:1984-1989; Seong et al., *J. Biol. Chem.* 277:32282-32293, 2002). The X-ray structure of the PBD-phosphopeptide complex now enables us to dissect the role of phospho-specific binding in this phenotype. In agreement with previous studies, we found that overexpression of a GFP-fusion of the Plk1 PBD in HeLa cells caused a dramatic increase in the population of cells in G2/M (60% for PBD-GFP- vs. 17% for GFP-expressing cells) (Figure 15). Importantly, this accumulation of mitotic cells was abolished by mutation of His-538 and Lys-540 (23% in G2/M). In addition, expression of the wild-type PBD-GFP construct induced aneuploidy in HeLa cells, evident as a peak of cells with DNA content >4N, in agreement with anti-Plk1 antibody microinjection studies reported by Lane and Nigg (Lane et al., *J. Cell. Biol.* 135:1701-1713, 1996). However, this effect was completely lost when the His/Lys pincer mutant was employed. The dominant negative effects strongly suggest that phosphopeptide-binding by the PBD in full-length Plk1 normally plays a role in both proper mitotic progression and in the establishment of a functional bipolar spindle to ensure equal chromosome segregation.

Phosphopeptide Binding to the PBD Stimulates Plk1 Kinase Activity

Lee and Erikson (Lee et al., *Mol. Cell. Biol.* 17:3408-3417, 1999) and Mundt et al. (Biochem. Biophys. Res. Commun. 239:377-385, 1997) observed that deletion of the C-terminus of Plk1 increased the kinase activity ~3-fold while Jang et al (Jang et al., *Proc. Natl. Acad. Sci. USA* 99:1984-1989, 2002) found that the

isolated Plk1 C-terminus interacts with and inhibits the activity of the isolated kinase domain towards the exogenous substrate casein. We observed the complementary result, namely that the kinase domain appears to inhibit phosphopeptide binding by the PBD. While the isolated Plk1 PBD binds strongly and specifically to pSer/pThr-containing peptides (Figure 13A), phosphopeptide binding by the PBD within full-length Plk1 is reduced at least 10-fold, and is considerably less phospho-dependent (Figure 16A, wt lanes). The phospho-specific binding component of full-length Plk1 is clearly mediated by the PBD (Figure 16A, compare wt pTP and TP lanes with H538A/K540M pTP and TP lanes). This suggested that a mutually inhibitory interaction exists between the Plk1 PBD and the kinase domain in full-length Plk1.

We wondered whether binding of the PBD to phosphopeptides was sufficient to relieve this intramolecular interaction and stimulate the activity of the kinase domain towards exogenous substrates. Baculovirally-produced Plk1 was therefore incubated with either the optimal PBD phosphopeptide or its non-phosphorylated counterpart and kinase activity towards casein measured by SDS-PAGE/autoradiography. As shown in Figure 16B, addition of the optimal PBD phosphopeptide increased Plk1 kinase activity by a factor of 2.6, while addition of the non-phosphorylated peptide had no effect. This result compares quite favourably with the ~ 2.5-fold stimulation of Src and Hck kinase activity that is observed when these full-length Src family kinases are incubated with their optimal SH2-binding phosphotyrosine peptides to relieve SH2-mediated inhibition of the kinase domain (Liu et al., *Oncogene* 8:1119-1126, 1993; Moarefi et al., *Nature* 385:650-653, 1997). Thus, our results for Plk1 suggested that binding of the PBD to primed phosphorylation sites not only serves to target the kinase domain to substrates but also simultaneously activates the kinase domain for substrate phosphorylation by relieving an inhibitory intramolecular interaction (Figure 16C).

In this study, we have elucidated a conserved phosphopeptide-binding motif that is recognized by the PBDs of all canonical members in the human Plk family, *Xenopus* Plx1 and *S. cerevesiae* Cdc5p. The high-resolution X-ray structure of the Polo-box domain bound to an optimal phosphothreonine peptide, provides a molecular rationale for motif selection, defines a new protein fold, and illustrates a unique mechanism for phospho-dependent ligand binding involving the participation of ordered solvent molecules, together with a conserved His/Lys pincer motif. We have identified a pSer/Thr-dependent mechanism of Plk activation in which intramolecular inhibition of the kinase by the PBD is relieved by PBD interaction with pre-phosphorylated binding targets.

Structural Definition of the Polo-box Domain: A General Phosphoprotein Recognition Module

Previous reports have described the presence of 1-3 Polo-boxes within the C-terminal regions of Polo-like kinases (Glover et al., *Genes Dev.* 12:3777-3787, 1998; Glover et al., *J. Cell. Biol.* 135:1681-1684, 1996; Nigg, *Curr. Opin. Cell. Biol.* 10:776-783, 1998; Seong et al., *J. Biol. Chem.* 277:32282-32293, 2002). Our structure now definitively shows that the PBD consists of two structurally homologous regions corresponding to two conserved Polo-box sequences. Phosphopeptide binding occurs at the interface of the two Polo-boxes, rationalizing both the observed 1:1 stoichiometry of PBD/ligand binding (Figure 5B) and the requirement for both Polo-boxes for efficient subcellular localization of Plk1 *in vivo* (Seong et al., *J. Biol. Chem.* 277:32282-32293, 2002). Polo-box Domains (PBDs) now join an expanding family of phosphoserine/phosphothreonine binding domains that includes 14-3-3 proteins, WW, FHA, WD40, and Smad MH2 domains (Yaffe et al., *Curr Opin Cell Biol* 13:131-138, 2001; Yaffe et al., *Structure* 9:R33-38, 2001). In contrast to other more ubiquitous phosphodependent binding modules, PBDs occur only in Polo-like kinases where they localize Plks to specific subcellular organelles and mitotic

structures (Jang et al., 2002; Lee et al., *Proc. Natl. Acad. Sci. USA* 95:9301-9306, 1998; (Lee et al., *Mol Cell Biol* 17, 3408-3417, 1999) and target the kinase to substrates that have been primed by prior phosphorylation.

Common Phosphopeptide Motif Selection by the PBD family

In higher eukaryotes, different Plk family members function at different points in the cell cycle (Donaldson et al., 2001; Glover *et al.*, *Genes Dev* 12:3777-3787, 1998; Glover *et al.*, J Cell Biol 135, 1681-1684, 1996; Ma *et al.*, Mol Cancer Res 1, 376-384, 2003; Nigg, Curr Opin Cell Biol 10:776-783, 1998) or play antagonistic roles in response to DNA damage (Bahassi *et al.*, *Oncogene* 21, 6633-6640, 2002; Smits et *al.*, *Nat Cell Biol* 2:672-676, 2000; Xie et al., *Cell Cycle* 1:424-429, 2002). Given the similarity in the selected motifs with a Ser-pSer/pThr-Pro/X core for these three proteins, potential mechanisms to separate Plks within a single organism achieve substrate specificity might include different substrate selectivities by their respective kinase domains, spatially and temporally restricted activation of Plks by upstream kinases, or the well documented cell-cycle regulation of Plk1 and 2 expression (Golsteyn et al., *Cell Sci* 107:1509-1517, 1994; Lee et al., 1995; Ma et al., *Mol Cancer Res* 1:376-384, 2003). One pathway in which such specificity must be vital is the DNA damage response, since Plk1 is inhibited by DNA damage (Smits et al., *Nat Cell Biol* 2:672-676, 2000), while Plk3 appears to be activated (Xie et al., *Cell Cycle* 1:424-429, 2002).

In addition to pThr –1 selectivity for serine, all PBDs that we have examined exhibit moderate specificity for proline at the pThr+1 position, emphasizing a central role for CDKs and other proline-directed kinases in priming substrates for Plk1 targeting. Several lines of evidence support this model. For example, maximal Plk1-induced activation and nuclear translocation of Cdc25 has been shown to require cyclin B coexpression (Toyoshima-Morimoto et al., *EMBO Rep.* 3:341-348, 2002). Furthermore, full reconstitution of purified APC activity requires prior synergistic phosphorylation of the APC by both Cdc2 and Plk1

(Golan et al., *J. Biol. Chem.* 277:15552-15557, 2002). Interestingly, the backbone torsion angles of the *trans*-proline in the Plk1-bound phosphopeptide are very similar to those of the equivalent Pro residue in the ternary cyclinA3/CDK2/peptide complex structure (Brown et al., *Nat. Cell. Biol.* 1:438-443, 1999). Thus, the conformation of the peptide in the PBD complex reflects not only the structural requirements for Plk interaction but also the requirements for the initial priming phosphorylation.

Nevertheless, a clear tolerance for residues other than proline demonstrates that other mitotic kinases may also serve as priming agents. In this regard, the NIMA-related kinase Fin1 has been recently shown to increase Plo1 affinity for spindle pole bodies in *S. pombe* (Grallert et al., *EMBO J.* 21:3096-3107, 2002). Identification of substrates for Plk family members, as well as the kinases involved in substrate priming is, therefore, important.

The Structural Basis of Phosphopeptide Binding

The PBD binds to phosphorylated epitopes in a way that is distinct from that observed previously in structures of other protein-phosphopeptide complexes (Yaffe et al., *Structure* 9:R33-38, 2001). These differences include the His/Lys pincer, a significant contribution from bridging water molecules and an unusual orientation of the pThr–1 residue that is directed toward the protein-binding surface. Although stereospecific, solvent-mediated binding has been described in other systems, 'solvent-bridged' interactions with the phosphoryl group have not been observed in any structures of protein-phosphopeptide complexes reported to date. Rather, the phospho moiety is always held by direct interactions, most often with highly conserved arginine side-chains (Eck et al., *Nature* 362:87-91, 1993; Waksman et al., *Nature* 358:646-653, 1992; Yaffe et al., *Structure* 9:R33-38, 2001). The importance of the His/Lys pincer in the Plk1 PBD structure is exemplified by our observations that its mutation abrogates phosphopeptide binding by the PBD *in vitro*, targeting of Plk1 to Cdc25C *in vivo*, and centrosomal

localization, as well as disrupt the ability of the isolated PBD to induce G2/M arrest and aberrant spindle function.

Structure-based sequence alignments (Figure 12B) show that the binding surface formed at the interface of the two Polo-boxes is the only totally conserved region in the PBD, further supporting our finding that the PBDs from different Plks generally select very similar optimal phosphopeptide binding motifs. Crucial hydrogen-bond interactions and van der Waals contacts with Trp-414 of Plk1 rationalize both the strong serine selection at the (pThr/pSer)-1 position and the fact that mutation of Trp-414 disrupts Plk1 function *in vivo* (Lee et al., *Proc. Natl. Acad. Sci. USA* 95:9301-9306, 1998). The absolute conservation of Trp-414 predicts that all family members should exhibit the same serine preference, and we now show that this is the case. Historically, the 10 amino acid sequence surrounding Trp-414 was considered the signature motif for the non-catalytic region of Polo-family kinases (Golsteyn et al., *Cell Sci.* 107:1509-1517, 1994).

Comparison of the Plk1 PBD and Sak Polo-box Structures

The Plk1 PBD and Sak Polo-box structures emphasize how related sequence motifs are able to form markedly different protein folds. Significant structural differences between homologous proteins have been observed only rarely and most prominently in the KH family of small RNA-binding domains (Grishin, Nucleic Acids Res. 29:638-643, 2001 and references therein). In this case, two distinct sub-families of structures are distinguishable by different topologies of α and β secondary structural elements although all share a related hydrophobic core and similar overall tertiary structure. The differences between the Plk1 PBD and Sak Polo-box are more extreme and emphasize how related sequence motifs are able to form markedly different protein folds. This, in turn, has considerable implications for both motif-based structure prediction and efforts to delineate biological function from structures of apparently homologous proteins.

How do these unexpected structural differences relate to PBD function in Plk1 and Polo-box function in Sak subfamily Plks? The grossly different architectures argue against conservation of the phosphoprotein-binding function since residues most intimately involved in phosphopeptide binding by Plk1 (e.g. His-538/Lys-540, Trp-414) are not conserved in Sak. Furthermore, examination of the electrostatic potential surface of the Sak Polo-box dimer shows no significant regions of positive charge (data not shown), a property otherwise common to phospho-dependent binding proteins.

A Model for Phospholigand-Induced Stimulation of Plk Kinase Activity

Two alternative models for intramolecular regulation of kinase activity by a phosphopeptide binding domain are exemplified by the mechanisms of SH2 domain-mediated inhibition in Src family kinases and SHP-family tyrosine phosphatases. In the Src-type model, the phosphopeptide binding cleft of the SH2 domain engages an internal phosphotyrosine motif at the C-terminus of the molecule to hold the kinase domain in an inactive conformation (Sicheri et al., *Nature* 385:602-609, 1997; Xu et al., *Nature* 385:595-602, 1997). We believe that Plk1 does not operate through this mechanism since it does not possess an internal optimal PBD binding site, and interaction of the PBD with the Plk1 kinase domain is not dependent on phosphorylation (Jang et al., *Proc. Natl. Acad. Sci. USA* 99:1984-1989, 2002). In fact, mutation of Thr-210 to Asp as a mimic of kinase activation loop phosphorylation, actually abolishes PBD binding (Jang et al., *Proc. Natl. Acad. Sci. USA* 99:1984-1989, 2002). Furthermore, mutation of Trp-414 in Polo-box 1 has been shown to have no effect on the basal level of Plk1 kinase activity (Lee et al., *Proc. Natl. Acad. Sci. USA* 95:9301-9306, 1998). Since mutations at this position disrupt phosphodependent PBD interactions, it would seem that kinase regulation occurs through a phospho-independent binding function of the PBD.

In the SHP2 model, binding of the back surface of the N-terminal SH2 domain to the phosphatase domain partially occludes the catalytic cleft and simultaneously deforms the SH2 domain's binding pocket to reduce its affinity for phosphopeptide ligands (Hof et al., *Cell* 92:441-450, 1998). This is entirely consistent with the reduced phosphopeptide binding that we observe for the PBD in the context of full-length Plk1 (Figure 8A, 8C). In the case of SHP2, high local concentrations of phosphotyrosine ligands are able to bind to the N-terminal SH2 domain, inducing a concomitant conformational rearrangement of the SH2 binding cleft that is transmitted to its phosphatase-interacting surface and releases the catalytically competent phosphatase domain. We believe Plks may be regulated by a related mechanism (Figure 8C). Some support for the SHP-like mechanism arises from our observation that the N-terminal Polo-box of one molecule in the crystallographic asymmetric unit that is not involved in extensive lattice contacts displays significantly higher temperature factors than its C-terminal counterpart (58Å^2 vs 37Å^2). This implies a rather dynamic association of the two Polo-boxes that is likely to be more pronounced in the absence of the phosphopeptide ligand. In our current model, binding of the phosphopeptide between the N- and C-terminal Polo motifs acts as a structural switch, stabilizing a conformation of the PBD that is inappropriate for association with the kinase domain. Subsequent T210D phosphorylation by upstream kinases would then serve to maintain the active state by preventing re-binding of the PBD to the kinase. Definitive proof of this mechanism will require the determination of structures of full-length Plk's and their complexes. This work is in progress.

It is clear that proper mitotic progression requires the highly regulated interplay between CDK's and a variety of other proteins kinases such as Aurora, NIMA, and Polo-like kinases, yet the molecular events that underlie the activity of many of these enzymes are largely unknown. The results of our integrated biochemical, structural and cell-biological approach now provide a framework within which the cellular function of the Polo-box motif can be understood. Plk1

is overexpressed in a variety of human tumors (Strebhardt et al., *JAMA* 283:479-480, 2000; Takai et al., *Cancer Lett.* 169:41-49, 2001), and down-regulation of human Plk1 has been shown to inhibit proliferation of cultured tumor cells (Elez et al., *Biochem. Biophys. Res. Commun.* 269:352-356, 2000; Liu et al., *Proc. Natl. Acad. Sci. USA* 100:5789-5794, 2003), suggesting that Plks are potentially important targets for therapeutic intervention. Here, we have shown that the Plk1 PBD binds to phosphorylated epitopes in a way that is distinct from any observed previously in structures of other protein-phosphopeptide complexes. The unique pattern of interactions with the Ser-pThr dipeptide suggest this motif may be employed as a useful template for the design of anti-proliferative inhibitors specifically directed against Polo-box domains. The experiments described above were carried out using the following methods.

Phospho-motif screen for phosphoserine/threonine binding domains

A phospho-motif-biased peptide library and its unphosphorylated counterpart were constructed as follows: biotin-Z-Gly-Z-Gly-Gly-Ala-X-X-B-X-pThr-Pro-X-X-X-X-Ala-Lys-Lys-Lys SEQ ID NO:~~40~~ 30 and biotin-Z-Gly-Z-Gly-Gly-Ala-X-X-B-X-Thr-Pro-X-X-X-X-Ala-Lys-Lys-Lys SEQ ID NO:~~41~~ 31, where pThr is phosphothreonine, Z indicates aminohexanoic acid, X denotes all amino acids except Cys, and B is a biased mixture of the amino acids P, L, I, V, F, M, W. Streptavidin beads (Pierce, 75pmol /μL gel) were incubated with a five-fold molar excess of each biotinylated library in 20 mM Tris/HCl (pH7.5), 125 mM NaCl, 0.5% NP-40, 1 mM EDTA and washed four times with the same buffer to remove unbound ligand. The bead-immobilized libraries (30 μL gel) were added to 6 μL of an *in vitro* translated [^{35}S]-labeled protein pool in 200 μL binding buffer (20 mM Tris/HCl (pH7.5), 125 mM NaCl, 0.5% NP-40, 1 mM EDTA, 1 mM DTT, 4 μg/mL pepstatin, 4 μg/mL aprotinin, 4 μg/mL leupeptin, 200 μM Na_3VO_4, 50 mM NaF). Each pool consisted of ~30 radiolabeled proteins produced by coupled *in vitro* transcription/translation (Promega) of a plasmid pool containing ~100 cDNA

clones from a unidirectional and oligo dT-primed human HeLa cell library in pCDNA3.1 (Kanai *et al.*, EMBOJ 19:6778-6791, 2000). After incubation at 4°C for 2-3 hours, the beads were rapidly washed four times with binding buffer prior to separation on SDS-PAGE (11.4%) and autoradiography. Positively scoring hits within pools were recognized as protein bands that interacted more strongly with the phosphorylated immobilized library than its unphosphorylated counterpart. Pools containing positively scoring clones were progressively subdivided using a 96-well format and re-screened for phospho-binding until single clones were isolated and identified by DNA sequencing.

Cloning, expression, and purification of Plk-1 PBD proteins

For deletion mapping of the PBD, C-terminal fragments of Plk-1 were generated by PCR and cloned into the EcoRI and XhoI sites of pCDNA3.1 (Invitrogen). For production of recombinant PBD as a GST fusion in bacteria, the 326-603 fragment of Plk-1 was ligated into the EcoRI and XhoI sites of pGEX-4T (Pharmacia), transformed into BL21, and induced in late log-phase cells at 37°C for 3.5 hours in the presence of 0.4 mM IPTG. For measurements of peptide binding affinity by ITC, GST-Plk-1 (326-603) was isolated from bacterial lysates using glutathione agarose, cleaved from GST using thrombin (10U/mL), and purified by anion exchange chromatography (Q Sepharose HP, Pharmacia).

Peptide Library Screening

Phosphothreonine- and phosphoserine-oriented degenerate peptide libraries containing the sequences Met-Ala-X-X-X-X-pThr-Pro-X-X-X-X-Ala-Lys-Lys-Lys SEQ ID NO:~~42~~ 46(theoretical degeneracy (td) =1.7×10^{10}), Met-Ala-X-X-X-X-pThr-X-X-X-X-Ala-Lys-Lys-Lys SEQ ID NO:~~43~~ 47(td = 1.7×10^{10}), Met-Ala-X-X-X-X-Ser-pThr-X-X-X-X-Ala-Lys-Lys-Lys SEQ ID NO:~~44~~ 48(td = 1.7×10^{10}), Met-Ala-X-X-X-pSer-Pro-X-X-X-Ala-Lys-Lys-Lys SEQ ID NO:~~45~~ 49(td = 4.7×10^{7}), Met-Ala-X-X-X-X-pSer-X-X-X-X-Ala-Lys-Lys-Lys SEQ ID NO:~~46~~

50(td = 1.7 x 10^{10}), and Met-Ala-X-X-X-X-Ser-pSer-X-X-X-X-Ala-Lys-Lys-Lys SEQ ID NO:~~47~~ 54(td = 1.7 x 10^{10}) were synthesized using N-α-FMOC-protected amino acids and standard BOP/HOBt coupling chemistry. Peptide library screening was performed using 100 µl of glutathione beads containing saturating amounts of GST-Plk-1 (residues 326-603) fusion protein (~1-1.5 mg) as described in Yaffe & Cantley (Methods Enzymol., 328:157-170, 2000). Beads were packed in a 1 mL column and incubated with 0.5 mg of the peptide library mixture for 10 minutes at room temperature in PBS (150 mM NaCl, 3 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.2). Unbound peptides were removed from the column by two rapid washes with PBS containing 0.5% NP-40 and two subsequent washes with PBS. Bound peptides were eluted with 30% acetic acid for 10 minutes at room temperature, lyophilized, resuspended in H_2O, and sequenced by automated Edman degradation on a Procise protein microsequencer. Selectivity values for each amino acid were determined by comparing the relative abundance (Mole percentage) of each amino acid at a particular sequencing cycle in the recovered peptides to that of each amino acid in the original peptide library mixture at the same position.

Isothermal Titration Calorimetry

Peptides were synthesized by solid phase technique with two C-terminal lysines to enhance solubility, purified by reverse phase HPLC following deprotection, and confirmed by MALDI-TOF 9 Matrix-assisted laser desorption/ionisation-time of flight mass spectrometry. Some peptides contained an additional tyrosine residue to facilitate concentration determination by optical absorbance. Calorimetry measurements were performed using a VP-ITC microcalorimeter (MicroCal Inc., Studio City, CA). Experiments involved 10µL injections of peptide solutions (150 µM-180 µM) into a sample cell containing 15µM Plk-1 PBD (residues 326-603) in 50 mM Tris/HCl (pH 8.1), 200 mM NaCl, 2 mM TCEP. Thirty injections were performed with a spacing of 240 s and a

reference power of 25 μCal/s. Binding isotherms were plotted and analyzed using Origin Software (MicroCal Inc. Studio City, CA).

Plk-1 PBD binding to cellular substrates

HeLa cells were arrested in interphase or G2/M by treatment with aphidicolin (5 μg/mL) or nocodazole (50 ng/mL), respectively, for 16 hours. Cells were lysed in 25 mM Tris/HCl (pH 7.5) containing 125 mM NaCl, 0.5% NP-40, 5 mM EDTA, 2 mM DTT, 4 μg/mL pepstatin, 4 μg/mL aprotinin, 4 μg/mL leupeptin, 1 mM Na_3VO_4, 50 mM NaF, and 1 μM microcystin, and 150 μgs of lysate incubated with 10 μL of glutathione agarose beads containing 2-5 μg of GST-Plk-1 (residues 326-603), GST-Pin1, or GST for 30 minutes at 4°C. Beads were washed four times with lysis buffer. Precipitated proteins were eluted in sample buffer and detected by blotting with monoclonal MPM-2 (Upstate Biotechnology, Inc.) or polyclonal anti-Cdc25C (Santa Cruz Biotechnology, Santa Cruz, California). For peptide competition experiments, GST-Plk-1 (residues 326-603) was immobilized on glutathionine beads and preincubated with 320 μM of PoloBoxtide-optimal, -8T, or -7V for 45 minutes at 4°C. For binding experiments involving mutant *cdc25C*, HeLa cells were transfected with wild-type and mutated versions of HA-tagged Cdc25C in pECE using Superfect (Qiagen, Valencia, CA). Nocodazole (50 ng/mL) was added seventeen hours after transfection and cells incubated for an additional 14 hours to arrest them in G2/M. Point mutations of Cdc25C were constructed using the QuickChange site-directed mutagenesis system (Stratagene) and verified by DNA sequencing.

Centrosomal localization of the Plk-1 PBD

U2OS cells were cultured in 8-well chamber slides and arrested at G2/M by treatment with nocodazole (50 ng/mL) for 14 hours. After rinsing with PBS, cells were incubated with 4 μM GST-Plk-1 PBD (residues 326-603) and Streptolysin-O (1 U/ml) in permeabilization buffer (25 mM HEPES (pH 7.9), 100 mM KCl, 3

mM NaCl, 200 mM sucrose, 20 mM NaF, 1 mM $NaOVO_4$) for 20 minutes at 37°C. Cells were fixed in 3% paraformaldehyde/2% sucrose for 10 minutes at room temperature and extracted with a 0.5% Triton X-100 solution containing 20 mM Tris-HCl (pH 7.4), 50 mM NaCl, 300 mM sucrose, and 3 mM $MgCl_2$ for 10 minutes at RT. Slides were stained with Alexa Fluor 488-conjugated anti-GST (Molecular Probes, Eugene, OR) and monoclonal anti-γ-tubulin (Sigma, St.Louis, MO) antibodies at 4°C overnight, then stained with a Texas Red conjugated anti-mouse secondary antibody for 60 minutes at room temperature and counterstained with 4 μg/ml DAPI. Cells were examined using a Nikon Eclipse E600 fluorescence microscope equipped with a SPOT RTcamera and software (Diagnostic Instruments, Livingston, Scotland). Images were analyzed using NIH Image. For peptide competition experiments, the GST-Plk-1 PBD solution was preincubated with 250 μM of its optimal phosphopeptide ligand (PoloBoxtide-optimal) or its unphosphorylated counterpart (PoloBoxtide-8T) for 15 minutes at room temperature prior to use.

To quantitate centrosomal localization of the GST-Plk-1 PBD relative to γ-tubulin, black and white images of single cells showing comparable overall intensity for Alexa Fluor and Texas Red were selected and scaled to an average grayscale value of 200 (1= white, 255=black). The normalized intensity of centrosome-specific Alexa Fluor 488 staining ($N.I._{AF488}$) or Texas Red staining ($N.I._{TR}$) above background was defined as $([I_{centrosome}-I_{cell}]/I_{cell})$ where $I_{centrosome}$ indicates the fluorescence intensity of either Alexa-Fluor 488 or Texas Red averaged over the centrosome and I_{cell} indicates the overall fluorescence intensity averaged over the entire cell. The relative GST-PBD/γ-tubulin specific staining was then calculated as $N.I._{AF488}/N.I._{TR}$.

Screens to Identify Novel Binding Pairs

Novel binding pairs can be identified by the methods of the invention. For example, phosphopeptides are generated that are biased to include MAP kinase

and Cell-cycle dependent kinase (Cdks) consensus phosphorylation sites (i.e., pSer-Pro), for use in screening for novel pSer-Pro binding polypeptides. Such a screen can be easily adapted to identify additional binding pairs. By taking advantage of the observation that protein kinases and phosphopeptide binding domains appear to co-evolve to recognize overlapping sequence motifs, phosphopeptides can be generated to follow specific protein kinase substrates. Thus, basophilic phosphopeptides having a core sequence including RXRSX[pS/pT] (SEQ ID NO: 55) (where R is arginine, pS is phosphoserine, pT is phosphothreonine, and X is any amino acid) can be used to identify novel binding partners dependent on the kinase, Akt. Other potential basophilic kinase substrates based on consensus phosphorylation sequences of protein kinase C (PKC), cAMP-dependent protein kinase (PKA), G-protein coupled receptor kinases such as β-ARK may also be used.

Several methods are known in the art to identify consensus kinase substrates, for example, in U.S.P.N. 5,532,167, U.S.P.N. 6,004,757, and WO 98/54577. Thus, degenerate phosphopeptides can be generated based on consensus kinase substrate peptide motifs. Exemplary kinase substrate peptide motifs that can be used include, without limitation, phosphopeptides derived from the consensus sequences of the serine/threonine kinases, Ca^{2+}/calmodulin dependent kinases (CaMKs), check point kinases (e.g. CHK, Rad53), myosin light chain kinases, DRAK, Trio, casein kinase 1, cell cycle dependent kinases (CDKs, e.g., Cdc2, Cdk4, Cdk6), glycogen synthase kinases (GSK), MAP kinases (e.g., Jnk, Erk, p38), STE family kinases (e.g., PAK, GCK/MAP4K), MAP kinase activated kinases (e.g., Mnk), eIF2α kinases (e.g., PERK, PKR, HRI, GCN2), Raf kinases (e.g., A-Raf, B-Raf), casein kinase II, aurora/Polo kinases, mixed lineage kinases (e.g., MLK1, -2, -3), AKAP, Activin-receptor like kinase (Kir4), CAK, Mos, Pim, and Ksr. Other kinase substrate-derived phosphopeptide sequences that can be used in the invention include those derived from the dual specificity kinases, WEE-1, MEKs, DYRKs, Tesk, Clk, HIPK, Mps-1, TSK, and C-TAK.

Dual specificity kinases also include polypeptides related to the lipid kinases FRAP, p110 PI3 Kinase, ATM, ATR, and DNA-PK.

Protein tyrosine kinase substrate peptide motifs can also be used in the invention and include phosphopeptides derived from the consensus substrate sequences of the receptor tyrosine kinases, which include the EGF-R family (e.g., EGF-R, Her2/Neu), PDGF-R, CSF-R, IGF-R, VEGF-R (e.g., Flk/Kdr, Flt), HGF-R (Met), NGF-R (e.g., TrkA, -B, -C), FGF-R, ROR, Tie-1, Tie-2/Tek, Eph (e.g., $EphA_{1-8}$, $EphB_{1-6}$), Rik, Ron, Ros, Ret, and from the cytoplasmic tyrosine kinases, which include, the Src family (e.g., Src, Lck, Lyn, Fyn, Hck, Yes), Abl, Csk, CTK, JAKs, FAK, ITK, BTK, Ack/Pyk, Tec, Tyk, Syk, Zap70, Fer, and Fes/Fps.

Binding pairs identified are not limited to those that include phosphopeptide binding domains. The methods of the invention may be used to identify virtually any peptide-binding domain in which the domain is identified by simultaneous screening of a protein/polypeptide expression library with a biased peptide library. For example, a screen for binding pairs is carried out to identify a peptide-binding domain, for example, a PDZ, SH3, or WW peptide binding domain. The "bait" peptide library contains a degenerate collection of peptides oriented around at least two or more fixed residues. A working example of such a screen is provided in the upper left panel of Figure 9B, where there is a band at ~24 kDa that binds the non-phosphopeptide library but not the phosphopeptide library., suggesting that it is specific for binding to BxTP motifs.

Cloning and Expression of PBD Proteins

C-terminal fragments of human Plk1 (residues 326-603), human Plk2 (residues 355-685), human Plk3 (residues 335-646), *Xenopus* Plx1 (residues 317-598), and *Saccharomyces cerevesiae* Cdc5p (residues 357-705) were amplified from IMAGE cDNA clones or directly from *S. cerevisiae* chromosomal DNA by PCR and ligated into suitably digested pGEX4T-3 or pGEX-6P1 (Pharmacia). Proteins were expressed in *E. coli* BL21(DE3) cells and purified by glutathione-

affinity chromatography. For measurements of peptide binding affinity and domain mapping experiments, proteins were cleaved from GST with either thrombin or viral protease 3C (Pharmacia-LKB, Peapack, NJ) and further purified by anion exchange chromatography (Q Sepharose HP, Pharmacia) or gel filtration (Superdex S-75, Pharmacia, Peapack, NJ).

Oriented Peptide Library Screening

Phosphothreonine-oriented degenerate peptide libraries containing the sequences Met-Ala-X-X-X-X-pThr-Pro-X-X-X-X-Ala-Lys-Lys-Lys SEQ ID NO:~~48~~ 46(theoretical degeneracy (td) =1.7×10^{10}) and Met-Ala-X-X-X-X-Ser-pThr-X-X-X-X-Ala-Lys-Lys-Lys SEQ ID NO:~~49~~ 48(td = 1.7×10^{10}) were synthesized using N-α-FMOC-protected amino acids and standard BOP/HOBt coupling chemistry. Peptide library screening was performed using 100 µl of glutathione beads containing saturating amounts (~1-1.5 mg) of GST-hPlk1 , GST-hPlk2, GST-hPlk3, GST-Plx1, or GST-Cdc5p as described previously (Yaffe et al., *Methods Enzymol* 328:157-170, 2000).

Peptide Binding Measurements

Peptides were synthesized by solid phase technique with two C-terminal lysines to enhance solubility. Some peptides contained an additional tyrosine residue to facilitate concentration determination by optical absorbance. Isothermal titration calorimetry was performed using a VP-ITC microcalorimeter (MicroCal Inc. Studio City, CA) by titration of 15-40µM solutions of PBD proteins with 30 x 10 µl injections of 150-400µM peptide in a starting volume of 1.4-2.0 ml. Binding isotherms were plotted and analyzed using Origin Software (MicroCal Inc. Studio City, CA). Binding of *in vitro* translated Plk1 PBD (wild type and mutants) to bead-immobilized pTP and TP peptide libraries was performed as described previously (Elia et al., Science 299:1228-1231, 2003). pTP and TP indicate the peptide libraries biotin-Z-Gly-Z-Gly-Gly-Ala-X-X-B-X-pThr-Pro-X-X-X-X-Ala-

Lys-Lys-Lys SEQ ID NO:~~50~~ 30 biotin-Z-Gly-Z-Gly-Gly-Ala-X-X-B-X-Thr-Pro-X-X-X-X-Ala-Lys-Lys-Lys SEQ ID NO:~~51~~ 31, respectively, where pThr is phosphothreonine, Z is aminohexanoic acid, X denotes all amino acids except Cys, and B is a biased mixture of the amino acids P, L, I, V, F, M, W.

Peptide Spot Array

An ABIMED peptide arrayer with a computer controlled Gilson diluter and liquid handling robot was used to synthesize peptides onto an amino-PEG cellulose membrane using N-α-FMOC-protected amino acids and DIC/HOBT coupling chemistry. The membrane was blocked in 5% milk/TBS-T (0.1%) for 2 hours at room temperature, incubated with 0.1 μM GST-Plk1 PBD (residues 326-603) in 5% milk, 50 mM Tris/HCl (pH 7.5), 150 mM NaCl, 2 mM EDTA, 2mM DTT for 1 hour at room temperature and washed with TBS-T (0.1%). It was then incubated with anti-GST conjugated HRP in 5% milk/ TBS-T (0.1%) for 1 hour at room temperature, washed with TBS-T (0.1%), and subjected to chemiluminescence.

Domain Mapping and Protein Purification

Limited proteolysis of Plk1 (residues 326-603) and Cdc5p were performed using trypsin or endoproteinase Glu-C (Promega). N- and C-terminal limits were determined by Edman sequencing and electrospray mass spectrometry. DNA sequences encoding the proteolytically-defined domains were amplified by PCR and cloned into pGEX-6P1 (Cdc5p) or a version modified to allow ligation-independent cloning that also permits fusion-protein cleavage with TEV protease (Stols et al., Pro. Expr. Purif. 25:8-15 2002) (SJS – unpublished data). Recombinant PBDs were then expressed and purified as above.

Crystallization and Structure Determination

For crystallization, the phosphopeptide MAGPMQSpTPLNGAYKK (SEQ ID NO:~~52~~ 56) was mixed with the Plk1 PBD fragment in a 1.5:1 stoichiometric excess and concentrated to ~0.2 mM in a buffer containing 20mM Tris.HCl pH 8.0/500mM NaCl, 1mM EDTA, 3mM DTT. Crystals were grown by microbatch methods at 18°C using a Douglas Instruments IMPAX 1-5 crystallization robot and belong to monoclinic space-group $P2_1$ (a=62.4Å, b=79.5Å, c=62.0Å, β=93.26°) with two complexes per asymmetric unit. Native data were collected on Station 14.1 at the SRS Daresbury using cryopreserved crystals at a temperature of 100°K. All data were reduced using the HKL suite of processing software (Otwinowski et al., *Meth. Enzymol.* 276:307-326, 1997). Phase information was derived from a three wavelength MAD experiment, using a single crystal of Se-methionine substituted PBD in complex with the phosphopeptide. Data for each wavelength were collected to a nominal 3.0Å spacing on Station 14.2 at the SRS, Daresbury, UK. Ten Se sites corresponding to five sites per monomer in the asymmetric unit were located, and the phases refined using SOLVE (Terwilliger *et al.*, *Acta Crystallogr. D. Biol. Crystallogr* 55:849-861, 1999). Phases were extended to ~2.5Å against the native data using real-space non-crystallographic symmetry averaging with solvent flattening in RESOLVE (Terwilliger *et al.*, *Acta Crystallogr. D. Biol. Crystallogr* 55:849-861, 1999). These maps were readily interpretable allowing a partial model of the PBD, together with seven residues of the phosphopeptide to be built using 'O' (Jones *et al.*, *Acta Crystallogr.* A 47:110-119, 1991). Subsequent refinement using native data to 1.9 Å was carried out using CNS (Brunger *et al.*, *Acta Crystallogr. D Biol. Crystallogr.* 54:905-921, 1998) and REFMAC 5.0-ARP/wARP from the CCP4 suite. A summary of statistics for the structure solution and refinement are shown in Table 5 (amino acids 20-241 of Plk1, SEQ ID NO: 109). Residues in bold: His538, Lys540, Trp414, and Leu491.

Table 5

Plk1-PBD.pdb

□□????----

□□□□□□□□□□□□□°˜□□îÇ□□CRYST1 62.352
79.518 61.993 90.00 93.26 90.00 P 1 21 1

SCALE1 0.016038 0.000000 0.000914 0.00000

SCALE2 0.000000 0.012576 0.000000 0.00000

SCALE3 0.000000 0.000000 0.016157 0.00000

ATOM	1	N	ALA	A	20	36.401	10.634	1.405	1.00	33.71	7	N
ATOM	2	CA	ALA	A	20	37.156	9.417	1.828	1.00	32.78	6	C
ATOM	3	CB	ALA	A	20	38.634	9.615	1.623	1.00	32.91	6	C
ATOM	4	C	ALA	A	20	36.862	9.066	3.284	1.00	31.86	6	C
ATOM	5	O	ALA	A	20	36.468	9.924	4.069	1.00	32.15	8	O
ATOM	6	N	LEU	A	21	37.062	7.804	-3.631	1.00	31.41	7	N
ATOM	7	CA	LEU	A	21	36.766	7.324	4.979	1.00	31.14	6	C
ATOM	8	CB	LEU	A	21	36.948	5.812	5.061	1.00	31.43	6	C
ATOM	9	CG	LEU	A	21	35.921	4.969	4.306	1.00	32.63	6	C
ATOM	10	CD1	LEU	A	21	36.274	3.499	4.379	1.00	32.84	6	C
ATOM	11	CD2	LEU	A	21	34.520	5.215	4.881	1.00	32.61	6	C
ATOM	12	C	LEU	A	21	37.637	8.010	6.018	1.00	31.08	6	C
ATOM	13	O	LEU	A	21	37.163	8.368	7.096	1.00	30.32	8	O
ATOM	14	N	SER	A	22	38.912	8.200	5.687	1.00	30.80	7	N
ATOM	15	CA	SER	A	22	39.852	8.854	6.589	1.00	31.39	6	C
ATOM	16	CB	SER	A	22	41.244	8.902	5.948	1.00	31.77	6	C
ATOM	17	OG	SER	A	22	42.200	8.363	6.833	1.00	35.33	8	O
ATOM	18	C	SER	A	22	39.378	10.264	6.935	1.00	31.00	6	C
ATOM	19	O	SER	A	22	39.403	10.669	8.094	1.00	30.62	8	O
ATOM	20	N	ASP	A	23	38.959	11.012	5.919	1.00	30.36	7	N
ATOM	21	CA	ASP	A	23	38.404	12.341	6.135	1.00	30.45	6	C
ATOM	22	CB	ASP	A	23	38.129	13.027	4.805	1.00	30.88	6	C
ATOM	23	CG	ASP	A	23	39.394	13.545	4.149	1.00	33.47	6	C
ATOM	24	OD1	ASP	A	23	40.452	13.591	4.819	1.00	34.01	8	O
ATOM	25	OD2	ASP	A	23	39.418	13.915	2.961	1.00	36.44	8	O
ATOM	26	C	ASP	A	23	37.126	12.293	6.974	1.00	29.75	6	C
ATOM	27	O	ASP	A	23	36.922	13.105	7.875	1.00	29.61	8	O
ATOM	28	N	MET	A	24	36.249	11.355	6.662	1.00	29.28	7	N
ATOM	29	CA	MET	A	24	35.024	11.224	7.432	1.00	28.49	6	C
ATOM	30	CB	MET	A	24	34.134	10.133	6.852	1.00	28.64	6	C
ATOM	31	CG	MET	A	24	32.785	10.050	7.547	1.00	29.20	6	C
ATOM	32	SD	MET	A	24	31.750	8.750	6.855	1.00	32.07	16	S
ATOM	33	CE	MET	A	24	31.461	9.420	5.196	1.00	29.51	6	C
ATOM	34	C	MET	A	24	35.335	10.920	8.897	1.00	28.13	6	C
ATOM	35	O	MET	A	24	34.693	11.451	9.793	1.00	27.58	8	O
ATOM	36	N	LEU	A	25	36.313	10.059	9.139	1.00	28.15	7	N
ATOM	37	CA	LEU	A	25	36.694	9.740	10.516	1.00	28.97	6	C
ATOM	38	CB	LEU	A	25	37.779	8.665	10.539	1.00	28.90	6	C
ATOM	39	CG	LEU	A	25	38.345	8.310	11.915	1.00	29.83	6	C
ATOM	40	CD1	LEU	A	25	37.224	7.836	12.841	1.00	29.82	6	C
ATOM	41	CD2	LEU	A	25	39.421	7.240	11.787	1.00	30.16	6	C
ATOM	42	C	LEU	A	25	37.158	10.988	11.261	1.00	28.74	6	C
ATOM	43	O	LEU	A	25	36.769	11.219	12.406	1.00	28.86	8	O
ATOM	44	N	GLN	A	26	37.971	11.812	10.602	1.00	28.76	7	N
ATOM	45	CA	GLN	A	26	38.480	13.026	11.236	1.00	28.88	6	C
ATOM	46	CB	GLN	A	26	39.463	13.764	10.309	1.00	29.72	6	C
ATOM	47	CG	GLN	A	26	40.667	12.948	9.920	1.00	33.49	6	C
ATOM	48	CD	GLN	A	26	41.649	13.722	9.050	1.00	38.46	6	C

ATOM	49	OE1	GLN	A	26	41.310	14.150	7.939	1.00	41.83	8	O
ATOM	50	NE2	GLN	A	26	42.864	13.898	9.546	1.00	39.82	7	N
ATOM	51	C	GLN	A	26	37.336	13.953	11.589	1.00	27.91	6	C
ATOM	52	O	GLN	A	26	37.307	14.528	12.675	1.00	27.39	8	O
ATOM	53	N	GLN	A	27	36.395	14.098	10.660	1.00	26.30	7	N
ATOM	54	CA	GLN	A	27	35.246	14.968	10.848	1.00	25.97	6	C
ATOM	55	CB	GLN	A	27	34.419	15.035	9.553	1.00	26.10	6	C
ATOM	56	CG	GLN	A	27	35.155	15.752	8.396	1.00	25.54	6	C
ATOM	57	CD	GLN	A	27	34.598	15.402	7.022	1.00	25.17	6	C
ATOM	58	OE1	GLN	A	27	33.521	14.808	6.903	1.00	25.56	8	O
ATOM	59	NE2	GLN	A	27	35.337	15.760	5.979	1.00	25.43	7	N
ATOM	60	C	GLN	A	27	34.366	14.489	12.005	1.00	25.75	6	C
ATOM	61	O	GLN	A	27	33.896	15.292	12.819	1.00	25.65	8	O
ATOM	62	N	LEU	A	28	34.135	13.184	12.055	1.00	25.72	7	N
ATOM	63	CA	LEU	A	28	33.317	12.590	13.121	1.00	26.50	6	C
ATOM	64	CB	LEU	A	28	32.975	11.134	12.778	1.00	26.13	6	C
ATOM	65	CG	LEU	A	28	31.914	10.996	11.687	1.00	26.32	6	C
ATOM	66	CD1	LEU	A	28	31.749	9.549	11.289	1.00	25.22	6	C
ATOM	67	CD2	LEU	A	28	30.580	11.563	12.173	1.00	26.83	6	C
ATOM	68	C	LEU	A	28	34.027	12.674	14.472	1.00	26.87	6	C
ATOM	69	O	LEU	A	28	33.417	13.019	15.488	1.00	27.36	8	O
ATOM	70	N	HIS	A	29	35.318	12.373	14.488	1.00	27.61	7	N
ATOM	71	CA	HIS	A	29	36.063	12.458	15.740	1.00	28.39	6	C
ATOM	72	CB	HIS	A	29	37.530	12.070	15.579	1.00	28.75	6	C
ATOM	73	CG	HIS	A	29	38.329	12.314	16.819	1.00	31.08	6	C
ATOM	74	ND1	HIS	A	29	38.125	11.598	17.978	1.00	31.37	7	N
ATOM	75	CE1	HIS	A	29	38.939	12.045	18.917	1.00	32.66	6	C
ATOM	76	NE2	HIS	A	29	39.647	13.041	18.417	1.00	31.82	7	N
ATOM	77	CD2	HIS	A	29	39.279	13.236	17.107	1.00	32.90	6	C
ATOM	78	C	HIS	A	29	35.989	13.870	16.283	1.00	28.68	6	C
ATOM	79	O	HIS	A	29	35.781	14.076	17.474	1.00	28.88	8	O
ATOM	80	N	SER	A	30	36.135	14.849	15.396	1.00	28.42	7	N
ATOM	81	CA	SER	A	30	36.122	16.241	15.810	1.00	28.05	6	C
ATOM	82	CB	SER	A	30	36.479	17.148	14.628	1.00	28.85	6	C
ATOM	83	OG	SER	A	30	36.538	18.498	15.053	1.00	30.19	8	O
ATOM	84	C	SER	A	30	34.811	16.685	16.452	1.00	27.75	6	C
ATOM	85	O	SER	A	30	34.812	17.298	17.521	1.00	26.57	8	O
ATOM	86	N	VAL	A	31	33.683	16.396	15.807	1.00	27.10	7	N
ATOM	87	CA	VAL	A	31	32.415	16.802	16.396	1.00	26.70	6	C
ATOM	88	CB	VAL	A	31	31.227	16.754	15.377	1.00	27.14	6	C
ATOM	89	CG1	VAL	A	31	31.125	15.396	14.732	1.00	26.15	6	C
ATOM	90	CG2	VAL	A	31	29.904	17.116	16.063	1.00	26.78	6	C
ATOM	91	C	VAL	A	31	32.095	15.979	17.658	1.00	26.15	6	C
ATOM	92	O	VAL	A	31	31.607	16.529	18.647	1.00	25.84	8	O
ATOM	93	N	ASN	A	32	32.375	14.677	17.632	1.00	25.70	7	N
ATOM	94	CA	ASN	A	32	32.050	13.827	18.789	1.00	25.94	6	C
ATOM	95	CB	ASN	A	32	32.251	12.348	18.486	1.00	25.11	6	C
ATOM	96	CG	ASN	A	32	31.242	11.800	17.473	1.00	25.06	6	C
ATOM	97	OD1	ASN	A	32	30.221	12.410	17.196	1.00	25.48	8	O
ATOM	98	ND2	ASN	A	32	31.550	10.645	16.924	1.00	24.23	7	N
ATOM	99	C	ASN	A	32	32.875	14.188	20.022	1.00	26.29	6	C
ATOM	100	O	ASN	A	32	32.378	14.153	21.142	1.00	26.53	8	O
ATOM	101	N	ALA	A	33	34.142	14.517	19.806	1.00	26.41	7	N
ATOM	102	CA	ALA	A	33	35.035	14.890	20.918	1.00	27.37	6	C
ATOM	103	CB	ALA	A	33	36.468	15.007	20.435	1.00	27.30	6	C
ATOM	104	C	ALA	A	33	34.595	16.187	21.584	1.00	27.63	6	C
ATOM	105	O	ALA	A	33	34.921	16.447	22.743	1.00	27.93	8	O

ATOM	106	N	SER	A	34	33.834	16.994	20.858	1.00	27.83	7	N
ATOM	107	CA	SER	A	34	33.347	18.251	21.397	1.00	28.41	6	C
ATOM	108	CB	SER	A	34	33.075	19.252	20.268	1.00	28.21	6	C
ATOM	109	OG	SER	A	34	31.807	19.031	19.670	1.00	27.66	8	O
ATOM	110	C	SER	A	34	32.105	18.089	22.290	1.00	28.78	6	C
ATOM	111	O	SER	A	34	31.643	19.069	22.882	1.00	28.93	8	O
ATOM	112	N	LYS	A	35	31.597	16.857	22.397	1.00	28.68	7	N
ATOM	113	CA	LYS	A	35	30.425	16.523	23.229	1.00	29.44	6	C
ATOM	114	CB	LYS	A	35	30.795	16.531	24.711	1.00	29.93	6	C
ATOM	115	CG	LYS	A	35	31.934	15.594	25.089	1.00	31.61	6	C
ATOM	116	CD	LYS	A	35	32.098	15.557	26.612	1.00	34.33	6	C
ATOM	117	CE	LYS	A	35	32.129	16.969	27.205	1.00	36.89	6	C
ATOM	118	NZ	LYS	A	35	32.313	16.996	28.699	1.00	39.71	7	N
ATOM	119	C	LYS	A	35	29.261	17.475	22.987	1.00	29.56	6	C
ATOM	120	O	LYS	A	35	28.822	18.180	23.894	1.00	29.19	8	O
ATOM	121	N	PRO	A	36	28.746	17.459	21.762	1.00	29.62	7	N
ATOM	122	CA	PRO	A	36	27.742	18.428	21.311	1.00	29.86	6	C
ATOM	123	CB	PRO	A	36	27.509	18.018	19.849	1.00	29.74	6	C
ATOM	124	CG	PRO	A	36	27.873	16.537	19.841	1.00	29.62	6	C
ATOM	125	CD	PRO	A	36	29.099	16.493	20.706	1.00	29.42	6	C
ATOM	126	C	PRO	A	36	26.424	18.435	22.079	1.00	30.15	6	C
ATOM	127	O	PRO	A	36	25.743	19.461	22.046	1.00	29.59	8	O
ATOM	128	N	SER	A	37	26.056	17.335	22.742	1.00	30.35	7	N
ATOM	129	CA	SER	A	37	24.796	17.310	23.482	1.00	30.73	6	C
ATOM	130	CB	SER	A	37	24.096	15.950	23.337	1.00	30.91	6	C
ATOM	131	OG	SER	A	37	24.788	14.951	24.059	1.00	30.05	8	O
ATOM	132	C	SER	A	37	24.988	17.653	24.963	1.00	31.78	6	C
ATOM	133	O	SER	A	37	24.028	17.746	25.717	1.00	31.53	8	O
ATOM	134	N	GLU	A	38	26.234	17.860	25.358	1.00	32.67	7	N
ATOM	135	CA	GLU	A	38	26.562	18.138	26.743	1.00	34.86	6	C
ATOM	136	CB	GLU	A	38	27.696	17.206	27.183	1.00	34.88	6	C
ATOM	137	CG	GLU	A	38	27.227	15.750	27.139	1.00	37.05	6	C
ATOM	138	CD	GLU	A	38	28.344	14.733	26.972	1.00	40.86	6	C
ATOM	139	OE1	GLU	A	38	29.059	14.473	27.960	1.00	40.91	8	O
ATOM	140	OE2	GLU	A	38	28.496	14.175	25.852	1.00	42.71	8	O
ATOM	141	C	GLU	A	38	26.875	19.622	26.931	1.00	35.55	6	C
ATOM	142	O	GLU	A	38	27.772	20.009	27.672	1.00	36.66	8	O
ATOM	143	N	ARG	A	39	26.091	20.442	26.244	1.00	36.48	7	N
ATOM	144	CA	ARG	A	39	26.224	21.887	26.286	1.00	37.25	6	C
ATOM	145	CB	ARG	A	39	26.169	22.456	24.865	1.00	37.41	6	C
ATOM	146	CG	ARG	A	39	27.186	21.845	23.903	1.00	38.11	6	C
ATOM	147	CD	ARG	A	39	28.580	22.457	24.002	1.00	38.15	6	C
ATOM	148	NE	ARG	A	39	29.559	21.725	23.204	1.00	38.73	7	N
ATOM	149	CZ	ARG	A	39	29.706	21.866	21.893	1.00	37.81	6	C
ATOM	150	NH1	ARG	A	39	28.941	22.718	21.228	1.00	37.87	7	N
ATOM	151	NH2	ARG	A	39	30.616	21.153	21.249	1.00	37.30	7	N
ATOM	152	C	ARG	A	39	25.058	22.433	27.079	1.00	37.01	6	C
ATOM	153	O	ARG	A	39	24.022	21.783	27.198	1.00	37.60	8	O
ATOM	154	N	GLY	A	40	25.207	23.636	27.607	1.00	36.91	7	N
ATOM	155	CA	GLY	A	40	24.123	24.232	28.365	1.00	36.46	6	C
ATOM	156	C	GLY	A	40	22.905	24.597	27.533	1.00	35.96	6	C
ATOM	157	O	GLY	A	40	21.769	24.456	27.975	1.00	37.22	8	O
ATOM	158	N	LEU	A	41	23.134	25.097	26.331	1.00	34.84	7	N
ATOM	159	CA	LEU	A	41	22.045	25.476	25.452	1.00	33.51	6	C
ATOM	160	CB	LEU	A	41	21.947	27.000	25.353	1.00	33.41	6	C
ATOM	161	CG	LEU	A	41	20.995	27.589	24.315	1.00	33.40	6	C
ATOM	162	CD1	LEU	A	41	19.561	27.317	24.719	1.00	33.71	6	C

ATOM	163	CD2	LEU	A	41	21.232	29.095	24.155	1.00	33.45	6	C
ATOM	164	C	LEU	A	41	22.353	24.903	24.085	1.00	33.07	6	C
ATOM	165	O	LEU	A	41	23.431	25.131	23.548	1.00	33.54	8	O
ATOM	166	N	VAL	A	42	21.419	24.146	23.532	1.00	32.14	7	N
ATOM	167	CA	VAL	A	42	21.617	23.570	22.208	1.00	31.38	6	C
ATOM	168	CB	VAL	A	42	21.141	22.101	22.170	1.00	31.56	6	C
ATOM	169	CG1	VAL	A	42	21.051	21.591	20.724	1.00	31.57	6	C
ATOM	170	CG2	VAL	A	42	22.086	21.241	22.991	1.00	30.77	6	C
ATOM	171	C	VAL	A	42	20.912	24.406	21.148	1.00	31.02	6	C
ATOM	172	O	VAL	A	42	19.771	24.820	21.340	1.00	31.11	8	O
ATOM	173	N	ARG	A	43	21.619	24.692	20.055	1.00	30.35	7	N
ATOM	174	CA	ARG	A	43	21.061	25.422	18.927	1.00	30.73	6	C
ATOM	175	CB	ARG	A	43	21.636	26.843	18.839	1.00	30.57	6	C
ATOM	176	CG	ARG	A	43	21.148	27.756	19.974	1.00	32.57	6	C
ATOM	177	CD	ARG	A	43	21.173	29.237	19.630	1.00	33.26	6	C
ATOM	178	NE	ARG	A	43	22.519	29.784	19.645	1.00	33.72	7	N
ATOM	179	CZ	ARG	A	43	22.929	30.792	18.880	1.00	33.34	6	C
ATOM	180	NH1	ARG	A	43	22.106	31.358	18.006	1.00	34.75	7	N
ATOM	181	NH2	ARG	A	43	24.169	31.228	18.986	1.00	34.75	7	N
ATOM	182	C	ARG	A	43	21.325	24.641	17.640	1.00	30.19	6	C
ATOM	183	O	ARG	A	43	22.000	25.114	16.731	1.00	30.53	8	O
ATOM	184	N	GLN	A	44	20.794	23.427	17.595	1.00	30.12	7	N
ATOM	185	CA	GLN	A	44	20.953	22.529	16.453	1.00	30.34	6	C
ATOM	186	CB	GLN	A	44	20.082	21.289	16.681	1.00	30.51	6	C
ATOM	187	CG	GLN	A	44	20.483	20.058	15.907	1.00	32.20	6	C
ATOM	188	CD	GLN	A	44	19.725	18.832	16.380	1.00	33.37	6	C
ATOM	189	OE1	GLN	A	44	19.786	18.488	17.549	1.00	34.97	8	O
ATOM	190	NE2	GLN	A	44	19.001	18.184	15.476	1.00	34.78	7	N
ATOM	191	C	GLN	A	44	20.571	23.181	15.132	1.00	29.89	6	C
ATOM	192	O	GLN	A	44	21.191	22.925	14.097	1.00	29.81	8	O
ATOM	193	N	ALA	A	45	19.543	24.022	15.155	1.00	29.90	7	N
ATOM	194	CA	ALA	A	45	19.060	24.636	13.920	1.00	30.12	6	C
ATOM	195	CB	ALA	A	45	17.754	25.393	14.155	1.00	30.53	6	C
ATOM	196	C	ALA	A	45	20.095	25.532	13.259	1.00	30.05	6	C
ATOM	197	O	ALA	A	45	20.044	25.762	12.054	1.00	29.93	8	O
ATOM	198	N	GLU	A	46	21.051	26.018	14.039	1.00	29.96	7	N
ATOM	199	CA	GLU	A	46	22.078	26.895	13.501	1.00	30.20	6	C
ATOM	200	CB	GLU	A	46	22.767	27.675	14.628	1.00	30.43	6	C
ATOM	201	CG	GLU	A	46	21.879	28.715	15.287	1.00	32.59	6	C
ATOM	202	CD	GLU	A	46	21.397	29.779	14.324	1.00	33.11	6	C
ATOM	203	OE1	GLU	A	46	22.124	30.091	13.354	1.00	34.58	8	O
ATOM	204	OE2	GLU	A	46	20.290	30.319	14.537	1.00	35.13	8	O
ATOM	205	C	GLU	A	46	23.112	26.121	12.687	1.00	29.91	6	C
ATOM	206	O	GLU	A	46	23.984	26.717	12.053	1.00	29.50	8	O
ATOM	207	N	ALA	A	47	23.007	24.795	12.699	1.00	29.19	7	N
ATOM	208	CA	ALA	A	47	23.948	23.958	11.957	1.00	28.42	6	C
ATOM	209	CB	ALA	A	47	24.384	22.768	12.804	1.00	28.21	6	C
ATOM	210	C	ALA	A	47	23.339	23.473	10.641	1.00	28.77	6	C
ATOM	211	O	ALA	A	47	24.010	22.818	9.843	1.00	27.99	8	O
ATOM	212	N	GLU	A	48	22.071	23.802	10.423	1.00	28.97	7	N
ATOM	213	CA	GLU	A	48	21.373	23.409	9.196	1.00	29.55	6	C
ATOM	214	CB	GLU	A	48	19.886	23.769	9.292	1.00	29.77	6	C
ATOM	215	CG	GLU	A	48	19.116	23.003	10.360	1.00	31.36	6	C
ATOM	216	CD	GLU	A	48	17.644	23.379	10.405	1.00	33.36	6	C
ATOM	217	OE1	GLU	A	48	17.200	24.140	9.524	1.00	33.45	8	O
ATOM	218	OE2	GLU	A	48	16.930	22.917	11.324	1.00	34.99	8	O
ATOM	219	C	GLU	A	48	21.975	24.062	7.949	1.00	29.78	6	C

```
ATOM    220  O   GLU A  48      22.231  25.265   7.921  1.00 28.44      8        O
ATOM    221  N   ASP A  49      22.188  23.260   6.911  1.00 30.40      7        N
ATOM    222  CA  ASP A  49      22.754  23.765   5.666  1.00 31.48      6        C
ATOM    223  CB  ASP A  49      24.268  23.563   5.663  1.00 32.13      6        C
ATOM    224  CG  ASP A  49      24.990  24.508   4.716  1.00 34.05      6        C
ATOM    225  OD1 ASP A  49      24.342  25.064   3.807  1.00 35.90      8        O
ATOM    226  OD2 ASP A  49      26.215  24.752   4.813  1.00 36.69      8        O
ATOM    227  C   ASP A  49      22.112  22.973   4.531  1.00 31.75      6        C
ATOM    228  O   ASP A  49      22.643  21.949   4.106  1.00 31.48      8        O
ATOM    229  N   PRO A  50      20.966  23.445   4.054  1.00 32.06      7        N
ATOM    230  CA  PRO A  50      20.224  22.748   2.994  1.00 32.74      6        C
ATOM    231  CB  PRO A  50      18.970  23.615   2.810  1.00 33.04      6        C
ATOM    232  CG  PRO A  50      18.897  24.474   4.020  1.00 32.80      6        C
ATOM    233  CD  PRO A  50      20.301  24.689   4.475  1.00 32.50      6        C
ATOM    234  C   PRO A  50      21.003  22.673   1.689  1.00 33.09      6        C
ATOM    235  O   PRO A  50      20.700  21.823   0.839  1.00 33.20      8        O
ATOM    236  N   ALA A  51      21.994  23.540   1.519  1.00 33.00      7        N
ATOM    237  CA  ALA A  51      22.807  23.506   0.305  1.00 32.95      6        C
ATOM    238  CB  ALA A  51      23.614  24.780   0.159  1.00 33.32      6        C
ATOM    239  C   ALA A  51      23.728  22.277   0.274  1.00 32.90      6        C
ATOM    240  O   ALA A  51      24.358  21.972  -0.748  1.00 32.85      8        O
ATOM    241  N   CYS A  52      23.791  21.571   1.395  1.00 31.55      7        N
ATOM    242  CA  CYS A  52      24.631  20.386   1.495  1.00 31.19      6        C
ATOM    243  CB  CYS A  52      25.420  20.413   2.799  1.00 31.18      6        C
ATOM    244  SG  CYS A  52      26.601  21.780   2.860  1.00 36.09     16        S
ATOM    245  C   CYS A  52      23.861  19.074   1.371  1.00 29.52      6        C
ATOM    246  O   CYS A  52      24.444  18.009   1.518  1.00 29.12      8        O
ATOM    247  N   ILE A  53      22.562  19.150   1.107  1.00 28.43      7        N
ATOM    248  CA  ILE A  53      21.753  17.939   0.942  1.00 28.06      6        C
ATOM    249  CB  ILE A  53      20.289  18.316   0.567  1.00 28.23      6        C
ATOM    250  CG1 ILE A  53      19.661  19.129   1.708  1.00 30.03      6        C
ATOM    251  CD1 ILE A  53      18.283  19.739   1.391  1.00 32.54      6        C
ATOM    252  CG2 ILE A  53      19.448  17.078   0.345  1.00 29.37      6        C
ATOM    253  C   ILE A  53      22.429  17.048  -0.109  1.00 27.44      6        C
ATOM    254  O   ILE A  53      22.935  17.550  -1.114  1.00 26.15      8        O
ATOM    255  N   PRO A  54      22.469  15.740   0.133  1.00 27.31      7        N
ATOM    256  CA  PRO A  54      23.141  14.815  -0.784  1.00 27.58      6        C
ATOM    257  CB  PRO A  54      23.057  13.458  -0.065  1.00 27.83      6        C
ATOM    258  CG  PRO A  54      22.513  13.719   1.293  1.00 28.07      6        C
ATOM    259  CD  PRO A  54      21.853  15.052   1.281  1.00 27.24      6        C
ATOM    260  C   PRO A  54      22.413  14.683  -2.117  1.00 27.78      6        C
ATOM    261  O   PRO A  54      21.196  14.891  -2.189  1.00 27.38      8        O
ATOM    262  N   ILE A  55      23.163  14.332  -3.154  1.00 27.67      7        N
ATOM    263  CA  ILE A  55      22.585  14.048  -4.454  1.00 28.32      6        C
ATOM    264  CB  ILE A  55      23.666  14.179  -5.548  1.00 28.85      6        C
ATOM    265  CG1 ILE A  55      24.293  15.579  -5.494  1.00 30.95      6        C
ATOM    266  CD1 ILE A  55      25.740  15.648  -5.965  1.00 33.86      6        C
ATOM    267  CG2 ILE A  55      23.054  13.925  -6.929  1.00 30.22      6        C
ATOM    268  C   ILE A  55      21.983  12.635  -4.455  1.00 27.58      6        C
ATOM    269  O   ILE A  55      20.922  12.400  -5.017  1.00 27.41      8        O
ATOM    270  N   PHE A  56      22.660  11.702  -3.790  1.00 26.32      7        N
ATOM    271  CA  PHE A  56      22.237  10.314  -3.766  1.00 25.72      6        C
ATOM    272  CB  PHE A  56      23.218   9.453  -4.581  1.00 25.89      6        C
ATOM    273  CG  PHE A  56      23.324   9.836  -6.034  1.00 27.67      6        C
ATOM    274  CD1 PHE A  56      24.429  10.528  -6.498  1.00 27.36      6        C
ATOM    275  CE1 PHE A  56      24.546  10.875  -7.834  1.00 28.92      6        C
ATOM    276  CZ  PHE A  56      23.556  10.552  -8.719  1.00 29.32      6        C
```

ATOM	277	CE2	PHE	A	56	22.437	9.856	-8.280	1.00	30.40	6	C
ATOM	278	CD2	PHE	A	56	22.327	9.496	-6.934	1.00	29.35	6	C
ATOM	279	C	PHE	A	56	22.226	9.718	-2.361	1.00	24.91	6	C
ATOM	280	O	PHE	A	56	23.036	10.085	-1.513	1.00	23.97	8	O
ATOM	281	N	TRP	A	57	21.312	8.781	-2.142	1.00	24.36	7	N
ATOM	282	CA	TRP	A	57	21.297	7.945	-0.942	1.00	24.65	6	C
ATOM	283	CB	TRP	A	57	20.622	8.641	0.260	1.00	24.01	6	C
ATOM	284	CG	TRP	A	57	19.175	9.036	0.010	1.00	25.03	6	C
ATOM	285	CD1	TRP	A	57	18.053	8.270	0.217	1.00	24.50	6	C
ATOM	286	NE1	TRP	A	57	16.924	8.976	-0.142	1.00	23.27	7	N
ATOM	287	CE2	TRP	A	57	17.298	10.223	-0.578	1.00	25.29	6	C
ATOM	288	CD2	TRP	A	57	18.705	10.298	-0.491	1.00	24.40	6	C
ATOM	289	CE3	TRP	A	57	19.337	11.486	-0.885	1.00	26.85	6	C
ATOM	290	CZ3	TRP	A	57	18.551	12.544	-1.342	1.00	28.68	6	C
ATOM	291	CH2	TRP	A	57	17.162	12.434	-1.409	1.00	27.81	6	C
ATOM	292	CZ2	TRP	A	57	16.516	11.289	-1.029	1.00	26.93	6	C
ATOM	293	C	TRP	A	57	20.572	6.649	-1.319	1.00	24.73	6	C
ATOM	294	O	TRP	A	57	19.945	6.577	-2.386	1.00	24.59	8	O
ATOM	295	N	VAL	A	58	20.684	5.630	-0.476	1.00	24.55	7	N
ATOM	296	CA	VAL	A	58	19.994	4.364	-0.702	1.00	24.83	6	C
ATOM	297	CB	VAL	A	58	20.741	3.191	-0.036	1.00	24.93	6	C
ATOM	298	CG1	VAL	A	58	19.939	1.887	-0.162	1.00	24.14	6	C
ATOM	299	CG2	VAL	A	58	22.109	3.016	-0.677	1.00	25.68	6	C
ATOM	300	C	VAL	A	58	18.544	4.447	-0.204	1.00	25.64	6	C
ATOM	301	O	VAL	A	58	18.296	4.723	0.976	1.00	25.76	8	O
ATOM	302	N	SER	A	59	17.597	4.220	-1.119	1.00	25.84	7	N
ATOM	303	CA	SER	A	59	16.170	4.341	-0.834	1.00	26.79	6	C
ATOM	304	CB	SER	A	59	15.449	4.907	-2.064	1.00	27.22	6	C
ATOM	305	OG	SER	A	59	15.274	6.298	-1.930	1.00	32.34	8	O
ATOM	306	C	SER	A	59	15.513	3.028	-0.437	1.00	26.12	6	C
ATOM	307	O	SER	A	59	14.527	3.018	0.314	1.00	25.45	8	O
ATOM	308	N	LYS	A	60	16.042	1.924	-0.965	1.00	25.71	7	N
ATOM	309	CA	LYS	A	60	15.524	0.592	-0.674	1.00	25.00	6	C
ATOM	310	CB	LYS	A	60	14.420	0.183	-1.665	1.00	25.95	6	C
ATOM	311	CG	LYS	A	60	13.282	1.185	-1.857	1.00	25.89	6	C
ATOM	312	CD	LYS	A	60	12.358	0.774	-3.030	1.00	27.60	6	C
ATOM	313	CE	LYS	A	60	11.199	1.774	-3.198	1.00	27.10	6	C
ATOM	314	NZ	LYS	A	60	10.221	1.300	-4.234	1.00	27.31	7	N
ATOM	315	C	LYS	A	60	16.697	-0.374	-0.821	1.00	24.95	6	C
ATOM	316	O	LYS	A	60	17.665	-0.065	-1.513	1.00	24.15	8	O
ATOM	**317**	**N**	**TRP**	**A**	**61**	**16.625**	**-1.510**	**-0.148**	**1.00**	**24.47**	**7**	**N**
ATOM	**318**	**CA**	**TRP**	**A**	**61**	**17.656**	**-2.541**	**-0.280**	**1.00**	**25.47**	**6**	**C**
ATOM	**319**	**CB**	**TRP**	**A**	**61**	**18.899**	**-2.210**	**0.566**	**1.00**	**25.28**	**6**	**C**
ATOM	**320**	**CG**	**TRP**	**A**	**61**	**18.610**	**-2.062**	**2.003**	**1.00**	**25.61**	**6**	**C**
ATOM	**321**	**CD1**	**TRP**	**A**	**61**	**18.356**	**-0.900**	**2.677**	**1.00**	**25.43**	**6**	**C**
ATOM	**322**	**NE1**	**TRP**	**A**	**61**	**18.136**	**-1.169**	**4.008**	**1.00**	**25.56**	**7**	**N**
ATOM	**323**	**CE2**	**TRP**	**A**	**61**	**18.229**	**-2.520**	**4.213**	**1.00**	**26.39**	**6**	**C**
ATOM	**324**	**CD2**	**TRP**	**A**	**61**	**18.538**	**-3.113**	**2.974**	**1.00**	**26.25**	**6**	**C**
ATOM	**325**	**CE3**	**TRP**	**A**	**61**	**18.698**	**-4.504**	**2.918**	**1.00**	**26.30**	**6**	**C**
ATOM	**326**	**CZ3**	**TRP**	**A**	**61**	**18.543**	**-5.245**	**4.080**	**1.00**	**27.94**	**6**	**C**
ATOM	**327**	**CH2**	**TRP**	**A**	**61**	**18.229**	**-4.624**	**5.299**	**1.00**	**27.03**	**6**	**C**
ATOM	**328**	**CZ2**	**TRP**	**A**	**61**	**18.077**	**-3.267**	**5.388**	**1.00**	**26.38**	**6**	**C**
ATOM	**329**	**C**	**TRP**	**A**	**61**	**17.102**	**-3.920**	**0.083**	**1.00**	**26.16**	**6**	**C**
ATOM	**330**	**O**	**TRP**	**A**	**61**	**16.158**	**-4.037**	**0.871**	**1.00**	**25.82**	**8**	**O**
ATOM	331	N	VAL	A	62	17.709	-4.958	-0.487	1.00	27.04	7	N
ATOM	332	CA	VAL	A	62	17.326	-6.346	-0.236	1.00	28.50	6	C
ATOM	333	CB	VAL	A	62	16.530	-6.937	-1.428	1.00	28.90	6	C

```
ATOM    334  CG1 VAL A  62      16.036  -8.339  -1.100  1.00 30.44      6      C
ATOM    335  CG2 VAL A  62      15.361  -6.042  -1.808  1.00 29.34      6      C
ATOM    336  C   VAL A  62      18.600  -7.169  -0.076  1.00 29.14      6      C
ATOM    337  O   VAL A  62      19.449  -7.171  -0.962  1.00 27.94      8      O
ATOM    338  N   ASP A  63      18.726  -7.869   1.048  1.00 30.63      7      N
ATOM    339  CA  ASP A  63      19.911  -8.672   1.335  1.00 31.95      6      C
ATOM    340  CB  ASP A  63      20.241  -8.584   2.824  1.00 31.90      6      C
ATOM    341  CG  ASP A  63      21.484  -9.378   3.215  1.00 32.72      6      C
ATOM    342  OD1 ASP A  63      22.047 -10.133   2.383  1.00 33.46      8      O
ATOM    343  OD2 ASP A  63      21.962  -9.306   4.361  1.00 31.63      8      O
ATOM    344  C   ASP A  63      19.736 -10.130   0.898  1.00 33.18      6      C
ATOM    345  O   ASP A  63      19.187 -10.958   1.632  1.00 33.29      8      O
ATOM    346  N   TYR A  64      20.206 -10.435  -0.302  1.00 34.24      7      N
ATOM    347  CA  TYR A  64      20.151 -11.797  -0.822  1.00 36.03      6      C
ATOM    348  CB  TYR A  64      19.507 -11.794  -2.203  1.00 36.45      6      C
ATOM    349  CG  TYR A  64      18.589 -12.965  -2.465  1.00 41.00      6      C
ATOM    350  CD1 TYR A  64      17.298 -12.767  -2.940  1.00 44.13      6      C
ATOM    351  CE1 TYR A  64      16.452 -13.837  -3.179  1.00 46.58      6      C
ATOM    352  CZ  TYR A  64      16.898 -15.125  -2.943  1.00 47.54      6      C
ATOM    353  OH  TYR A  64      16.068 -16.200  -3.177  1.00 50.35      8      O
ATOM    354  CE2 TYR A  64      18.175 -15.346  -2.471  1.00 46.59      6      C
ATOM    355  CD2 TYR A  64      19.011 -14.270  -2.233  1.00 44.35      6      C
ATOM    356  C   TYR A  64      21.575 -12.330  -0.902  1.00 35.84      6      C
ATOM    357  O   TYR A  64      21.925 -13.065  -1.823  1.00 35.73      8      O
ATOM    358  N   SER A  65      22.398 -11.950   0.070  1.00 36.70      7      N
ATOM    359  CA  SER A  65      23.818 -12.299   0.047  1.00 37.40      6      C
ATOM    360  CB  SER A  65      24.627 -11.399   0.979  1.00 37.31      6      C
ATOM    361  OG  SER A  65      24.385 -11.718   2.333  1.00 37.43      8      O
ATOM    362  C   SER A  65      24.063 -13.766   0.369  1.00 38.51      6      C
ATOM    363  O   SER A  65      25.198 -14.229   0.368  1.00 38.36      8      O
ATOM    364  N   ASP A  66      22.979 -14.478   0.649  1.00 39.68      7      N
ATOM    365  CA  ASP A  66      23.006 -15.906   0.892  1.00 40.94      6      C
ATOM    366  CB  ASP A  66      21.603 -16.348   1.317  1.00 41.90      6      C
ATOM    367  CG  ASP A  66      21.621 -17.525   2.252  1.00 45.04      6      C
ATOM    368  OD1 ASP A  66      22.727 -17.914   2.693  1.00 49.21      8      O
ATOM    369  OD2 ASP A  66      20.575 -18.128   2.603  1.00 48.82      8      O
ATOM    370  C   ASP A  66      23.349 -16.621  -0.403  1.00 40.56      6      C
ATOM    371  O   ASP A  66      23.967 -17.700  -0.396  1.00 40.67      8      O
ATOM    372  N   LYS A  67      22.945 -16.018  -1.518  1.00 39.66      7      N
ATOM    373  CA  LYS A  67      23.078 -16.670  -2.819  1.00 38.94      6      C
ATOM    374  CB  LYS A  67      21.744 -17.344  -3.183  1.00 39.57      6      C
ATOM    375  CG  LYS A  67      21.368 -18.486  -2.245  1.00 41.78      6      C
ATOM    376  CD  LYS A  67      19.921 -18.941  -2.419  1.00 45.87      6      C
ATOM    377  CE  LYS A  67      19.497 -19.841  -1.248  1.00 48.20      6      C
ATOM    378  NZ  LYS A  67      18.171 -20.498  -1.466  1.00 49.72      7      N
ATOM    379  C   LYS A  67      23.540 -15.783  -3.979  1.00 37.58      6      C
ATOM    380  O   LYS A  67      24.265 -16.250  -4.857  1.00 37.13      8      O
ATOM    381  N   TYR A  68      23.132 -14.514  -3.984  1.00 35.65      7      N
ATOM    382  CA  TYR A  68      23.457 -13.624  -5.097  1.00 34.39      6      C
ATOM    383  CB  TYR A  68      22.183 -13.231  -5.841  1.00 34.80      6      C
ATOM    384  CG  TYR A  68      21.317 -14.414  -6.216  1.00 37.04      6      C
ATOM    385  CD1 TYR A  68      20.096 -14.625  -5.593  1.00 39.02      6      C
ATOM    386  CE1 TYR A  68      19.295 -15.708  -5.926  1.00 40.75      6      C
ATOM    387  CZ  TYR A  68      19.710 -16.596  -6.895  1.00 41.85      6      C
ATOM    388  OH  TYR A  68      18.897 -17.663  -7.223  1.00 44.07      8      O
ATOM    389  CE2 TYR A  68      20.922 -16.411  -7.538  1.00 40.85      6      C
ATOM    390  CD2 TYR A  68      21.723 -15.322  -7.192  1.00 38.99      6      C
```

ATOM	391	C	TYR	A	68	24.236	-12.365	-4.720	1.00	32.93	6	C
ATOM	392	O	TYR	A	68	25.242	-12.032	-5.354	1.00	31.97	8	O
ATOM	393	N	GLY	A	69	23.761	-11.655	-3.705	1.00	31.18	7	N
ATOM	394	CA	GLY	A	69	24.428	-10.437	-3.289	1.00	29.97	6	C
ATOM	395	C	GLY	A	69	23.447	-9.452	-2.675	1.00	29.19	6	C
ATOM	396	O	GLY	A	69	22.369	-9.840	-2.229	1.00	28.66	8	O
ATOM	397	N	LEU	A	70	23.831	-8.182	-2.652	1.00	28.13	7	N
ATOM	398	CA	LEU	A	70	22.985	-7.148	-2.089	1.00	27.85	6	C
ATOM	399	CB	LEU	A	70	23.752	-6.354	-1.044	1.00	27.96	6	C
ATOM	400	CG	LEU	A	70	22.788	-5.400	-0.333	1.00	29.94	6	C
ATOM	401	CD1	LEU	A	70	22.772	-5.653	1.159	1.00	29.95	6	C
ATOM	402	CD2	LEU	A	70	23.013	-3.946	-0.700	1.00	30.21	6	C
ATOM	403	C	LEU	A	70	22.467	-6.215	-3.163	1.00	27.20	6	C
ATOM	404	O	LEU	A	70	23.244	-5.529	-3.811	1.00	27.61	8	O
ATOM	405	N	GLY	A	71	21.148	-6.185	-3.337	1.00	26.88	7	N
ATOM	406	CA	GLY	A	71	20.513	-5.309	-4.312	1.00	26.41	6	C
ATOM	407	C	GLY	A	71	19.989	-4.043	-3.641	1.00	25.86	6	C
ATOM	408	O	GLY	A	71	19.567	-4.078	-2.488	1.00	25.29	8	O
ATOM	409	N	TYR	A	72	20.013	-2.924	-4.350	1.00	25.62	7	N
ATOM	410	CA	TYR	A	72	19.566	-1.666	-3.745	1.00	25.82	6	C
ATOM	411	CB	TYR	A	72	20.731	-1.032	-2.949	1.00	25.61	6	C
ATOM	412	CG	TYR	A	72	21.915	-0.698	-3.831	1.00	25.97	6	C
ATOM	413	CD1	TYR	A	72	21.987	0.524	-4.483	1.00	26.37	6	C
ATOM	414	CE1	TYR	A	72	23.041	0.830	-5.313	1.00	26.23	6	C
ATOM	415	CZ	TYR	A	72	24.049	-0.107	-5.506	1.00	26.67	6	C
ATOM	416	OH	TYR	A	72	25.095	0.210	-6.327	1.00	27.29	8	O
ATOM	417	CE2	TYR	A	72	24.005	-1.330	-4.868	1.00	24.54	6	C
ATOM	418	CD2	TYR	A	72	22.941	-1.626	-4.044	1.00	24.92	6	C
ATOM	419	C	TYR	A	72	19.026	-0.684	-4.790	1.00	25.98	6	C
ATOM	420	O	TYR	A	72	19.272	-0.829	-5.989	1.00	25.98	8	O
ATOM	421	N	GLN	A	73	18.243	0.287	-4.325	1.00	25.67	7	N
ATOM	422	CA	GLN	A	73	17.746	1.349	-5.172	1.00	26.04	6	C
ATOM	423	CB	GLN	A	73	16.218	1.439	-5.100	1.00	26.48	6	C
ATOM	424	CG	GLN	A	73	15.642	2.564	-5.972	1.00	27.53	6	C
ATOM	425	CD	GLN	A	73	14.208	2.947	-5.601	1.00	30.17	6	C
ATOM	426	OE1	GLN	A	73	13.944	3.366	-4.474	1.00	28.93	8	O
ATOM	427	NE2	GLN	A	73	13.288	2.825	-6.560	1.00	29.51	7	N
ATOM	428	C	GLN	A	73	18.329	2.675	-4.660	1.00	25.84	6	C
ATOM	429	O	GLN	A	73	18.397	2.899	-3.447	1.00	25.54	8	O
ATOM	430	N	LEU	A	74	18.779	3.528	-5.566	1.00	25.94	7	N
ATOM	431	CA	LEU	A	74	19.165	4.877	-5.161	1.00	25.91	6	C
ATOM	432	CB	LEU	A	74	20.345	5.427	-5.966	1.00	25.32	6	C
ATOM	433	CG	LEU	A	74	21.679	4.666	-5.878	1.00	26.95	6	C
ATOM	434	CD1	LEU	A	74	22.775	5.388	-6.660	1.00	26.40	6	C
ATOM	435	CD2	LEU	A	74	22.115	4.443	-4.424	1.00	27.89	6	C
ATOM	436	C	LEU	A	74	17.936	5.775	-5.292	1.00	26.16	6	C
ATOM	437	O	LEU	A	74	16.957	5.420	-5.958	1.00	25.70	8	O
ATOM	438	N	CYS	A	75	17.994	6.937	-4.656	1.00	25.94	7	N
ATOM	439	CA	CYS	A	75	16.862	7.872	-4.631	1.00	27.22	6	C
ATOM	440	CB	CYS	A	75	17.205	9.034	-3.697	1.00	26.98	6	C
ATOM	441	SG	CYS	A	75	18.557	10.044	-4.311	1.00	28.44	16	S
ATOM	442	C	CYS	A	75	16.402	8.414	-5.998	1.00	27.82	6	C
ATOM	443	O	CYS	A	75	15.287	8.945	-6.120	1.00	28.35	8	O
ATOM	444	N	ASP	A	76	17.253	8.294	-7.014	1.00	28.30	7	N
ATOM	445	CA	ASP	A	76	16.927	8.745	-8.375	1.00	29.16	6	C
ATOM	446	CB	ASP	A	76	18.199	9.183	-9.100	1.00	29.04	6	C
ATOM	447	CG	ASP	A	76	19.061	7.999	-9.514	1.00	30.05	6	C

ATOM	448	OD1	ASP	A	76	19.836	8.131	-10.488	1.00	31.11	8	O
ATOM	449	OD2	ASP	A	76	19.018	6.897	-8.925	1.00	28.83	8	O
ATOM	450	C	ASP	A	76	16.240	7.651	-9.197	1.00	29.24	6	C
ATOM	451	O	ASP	A	76	16.018	7.813	-10.403	1.00	29.76	8	O
ATOM	452	N	ASN	A	77	15.929	6.541	-8.534	1.00	29.36	7	N
ATOM	453	CA	ASN	A	77	15.269	5.367	-9.117	1.00	29.49	6	C
ATOM	454	CB	ASN	A	77	14.035	5.757	-9.938	1.00	30.31	6	C
ATOM	455	CG	ASN	A	77	13.039	6.559	-9.121	1.00	32.03	6	C
ATOM	456	OD1	ASN	A	77	12.765	6.237	-7.960	1.00	32.48	8	O
ATOM	457	ND2	ASN	A	77	12.510	7.618	-9.713	1.00	34.72	7	N
ATOM	458	C	ASN	A	77	16.169	4.355	-9.860	1.00	28.92	6	C
ATOM	459	O	ASN	A	77	15.682	3.323	-10.343	1.00	28.69	8	O
ATOM	460	N	SER	A	78	17.465	4.652	-9.943	1.00	28.22	7	N
ATOM	461	CA	SER	A	78	18.426	3.689	-10.479	1.00	27.62	6	C
ATOM	462	CB	SER	A	78	19.815	4.313	-10.669	1.00	27.53	6	C
ATOM	463	OG	SER	A	78	20.396	4.720	-9.427	1.00	28.06	8	O
ATOM	464	C	SER	A	78	18.517	2.540	-9.485	1.00	27.25	6	C
ATOM	465	O	SER	A	78	18.196	2.705	-8.312	1.00	26.90	8	O
ATOM	466	N	VAL	A	79	18.946	1.370	-9.947	1.00	27.24	7	N
ATOM	467	CA	VAL	A	79	19.108	0.237	-9.054	1.00	27.17	6	C
ATOM	468	CB	VAL	A	79	18.043	-0.866	-9.292	1.00	27.70	6	C
ATOM	469	CG1	VAL	A	79	16.627	-0.363	-8.933	1.00	27.98	6	C
ATOM	470	CG2	VAL	A	79	18.100	-1.369	-10.720	1.00	28.75	6	C
ATOM	471	C	VAL	A	79	20.516	-0.323	-9.224	1.00	27.02	6	C
ATOM	472	O	VAL	A	79	21.173	-0.094	-10.238	1.00	26.92	8	O
ATOM	473	N	GLY	A	80	20.993	-1.038	-8.220	1.00	26.41	7	N
ATOM	474	CA	GLY	A	80	22.324	-1.591	-8.302	1.00	26.60	6	C
ATOM	475	C	GLY	A	80	22.419	-2.870	-7.513	1.00	26.72	6	C
ATOM	476	O	GLY	A	80	21.537	-3.203	-6.730	1.00	26.68	8	O
ATOM	477	N	VAL	A	81	23.496	-3.602	-7.732	1.00	27.22	7	N
ATOM	478	CA	VAL	A	81	23.731	-4.813	-6.976	1.00	27.84	6	C
ATOM	479	CB	VAL	A	81	23.230	-6.069	-7.704	1.00	27.76	6	C
ATOM	480	CG1	VAL	A	81	23.847	-6.152	-9.075	1.00	30.13	6	C
ATOM	481	CG2	VAL	A	81	23.561	-7.335	-6.893	1.00	27.79	6	C
ATOM	482	C	VAL	A	81	25.218	-4.962	-6.729	1.00	27.52	6	C
ATOM	483	O	VAL	A	81	26.046	-4.662	-7.594	1.00	27.69	8	O
ATOM	484	N	LEU	A	82	25.551	-5.387	-5.524	1.00	26.88	7	N
ATOM	485	CA	LEU	A	82	26.921	-5.726	-5.211	1.00	27.04	6	C
ATOM	486	CB	LEU	A	82	27.370	-5.067	-3.907	1.00	26.63	6	C
ATOM	487	CG	LEU	A	82	28.677	-5.575	-3.286	1.00	29.59	6	C
ATOM	488	CD1	LEU	A	82	29.730	-5.961	-4.330	1.00	31.06	6	C
ATOM	489	CD2	LEU	A	82	29.224	-4.575	-2.259	1.00	29.35	6	C
ATOM	490	C	LEU	A	82	26.851	-7.239	-5.115	1.00	26.34	6	C
ATOM	491	O	LEU	A	82	26.353	-7.799	-4.127	1.00	26.20	8	O
ATOM	492	N	PHE	A	83	27.311	-7.898	-6.177	1.00	26.15	7	N
ATOM	493	CA	PHE	A	83	27.266	-9.350	-6.261	1.00	26.17	6	C
ATOM	494	CB	PHE	A	83	27.536	-9.809	-7.699	1.00	25.60	6	C
ATOM	495	CG	PHE	A	83	26.433	-9.467	-8.678	1.00	26.75	6	C
ATOM	496	CD1	PHE	A	83	26.644	-8.533	-9.676	1.00	26.59	6	C
ATOM	497	CE1	PHE	A	83	25.652	-8.235	-10.592	1.00	27.88	6	C
ATOM	498	CZ	PHE	A	83	24.432	-8.871	-10.510	1.00	28.25	6	C
ATOM	499	CE2	PHE	A	83	24.211	-9.810	-9.527	1.00	28.69	6	C
ATOM	500	CD2	PHE	A	83	25.209	-10.097	-8.607	1.00	26.35	6	C
ATOM	501	C	PHE	A	83	28.275	-10.036	-5.338	1.00	26.24	6	C
ATOM	502	O	PHE	A	83	29.308	-9.478	-4.993	1.00	25.94	8	O
ATOM	503	N	ASN	A	84	27.981	-11.277	-4.982	1.00	26.65	7	N
ATOM	504	CA	ASN	A	84	28.866	-12.062	-4.122	1.00	27.40	6	C

ATOM	505	CB	ASN	A	84	28.232	-13.424	-3.830	1.00	27.75	6	C
ATOM	506	CG	ASN	A	84	27.168	-13.348	-2.769	1.00	30.05	6	C
ATOM	507	OD1	ASN	A	84	26.839	-12.261	-2.277	1.00	29.88	8	O
ATOM	508	ND2	ASN	A	84	26.623	-14.504	-2.394	1.00	29.43	7	N
ATOM	509	C	ASN	A	84	30.275	-12.275	-4.676	1.00	27.09	6	C
ATOM	510	O	ASN	A	84	31.189	-12.621	-3.929	1.00	27.36	8	O
ATOM	511	N	ASN	A	85	30.449	-12.093	-5.979	1.00	26.58	7	N
ATOM	512	CA	ASN	A	85	31.763	-12.223	-6.588	1.00	26.96	6	C
ATOM	513	CB	ASN	A	85	31.644	-12.772	-8.009	1.00	27.42	6	C
ATOM	514	CG	ASN	A	85	30.946	-11.795	-8.950	1.00	26.92	6	C
ATOM	515	OD1	ASN	A	85	30.513	-10.720	-8.539	1.00	29.27	8	O
ATOM	516	ND2	ASN	A	85	30.851	-12.158	-10.211	1.00	26.62	7	N
ATOM	517	C	ASN	A	85	32.534	-10.898	-6.618	1.00	26.95	6	C
ATOM	518	O	ASN	A	85	33.563	-10.794	-7.273	1.00	26.21	8	O
ATOM	519	N	SER	A	86	32.010	-9.893	-5.919	1.00	27.52	7	N
ATOM	520	CA	SER	A	86	32.627	-8.563	-5.827	1.00	27.99	6	C
ATOM	521	CB	SER	A	86	34.076	-8.638	-5.354	1.00	28.25	6	C
ATOM	522	OG	SER	A	86	34.114	-9.058	-4.012	1.00	31.12	8	O
ATOM	523	C	SER	A	86	32.544	-7.699	-7.079	1.00	27.67	6	C
ATOM	524	O	SER	A	86	33.267	-6.704	-7.197	1.00	28.16	8	O
ATOM	525	N	THR	A	87	31.690	-8.067	-8.018	1.00	26.97	7	N
ATOM	526	CA	THR	A	87	31.472	-7.178	-9.157	1.00	26.07	6	C
ATOM	527	CB	THR	A	87	31.330	-7.954	-10.466	1.00	25.82	6	C
ATOM	528	OG1	THR	A	87	30.155	-8.782	-10.416	1.00	23.37	8	O
ATOM	529	CG2	THR	A	87	32.496	-8.954	-10.633	1.00	24.35	6	C
ATOM	530	C	THR	A	87	30.203	-6.416	-8.844	1.00	26.76	6	C
ATOM	531	O	THR	A	87	29.409	-6.851	-8.002	1.00	26.49	8	O
ATOM	532	N	ARG	A	88	30.007	-5.282	-9.504	1.00	26.58	7	N
ATOM	533	CA	ARG	A	88	28.807	-4.486	-9.277	1.00	27.64	6	C
ATOM	534	CB	ARG	A	88	29.136	-3.257	-8.418	1.00	28.83	6	C
ATOM	535	CG	ARG	A	88	30.493	-3.375	-7.687	1.00	32.71	6	C
ATOM	536	CD	ARG	A	88	30.554	-2.751	-6.330	1.00	38.97	6	C
ATOM	537	NE	ARG	A	88	31.926	-2.457	-5.938	1.00	42.42	7	N
ATOM	538	CZ	ARG	A	88	32.537	-2.966	-4.878	1.00	44.46	6	C
ATOM	539	NH1	ARG	A	88	31.909	-3.806	-4.081	1.00	47.01	7	N
ATOM	540	NH2	ARG	A	88	33.789	-2.628	-4.607	1.00	46.70	7	N
ATOM	541	C	ARG	A	88	28.184	-4.088	-10.610	1.00	27.27	6	C
ATOM	542	O	ARG	A	88	28.891	-3.834	-11.582	1.00	26.57	8	O
ATOM	543	N	LEU	A	89	26.859	-4.039	-10.647	1.00	27.53	7	N
ATOM	544	CA	LEU	A	89	26.136	-3.680	-11.859	1.00	27.62	6	C
ATOM	545	CB	LEU	A	89	25.461	-4.914	-12.447	1.00	27.92	6	C
ATOM	546	CG	LEU	A	89	24.688	-4.784	-13.759	1.00	28.76	6	C
ATOM	547	CD1	LEU	A	89	25.579	-4.250	-14.882	1.00	29.33	6	C
ATOM	548	CD2	LEU	A	89	24.090	-6.152	-14.140	1.00	30.55	6	C
ATOM	549	C	LEU	A	89	25.083	-2.647	-11.492	1.00	27.77	6	C
ATOM	550	O	LEU	A	89	24.386	-2.790	-10.482	1.00	27.57	8	O
ATOM	551	N	ILE	A	90	24.982	-1.604	-12.308	1.00	27.83	7	N
ATOM	552	CA	ILE	A	90	24.023	-0.532	-12.076	1.00	28.43	6	C
ATOM	553	CB	ILE	A	90	24.763	0.807	-11.845	1.00	28.80	6	C
ATOM	554	CG1	ILE	A	90	25.556	0.776	-10.538	1.00	30.29	6	C
ATOM	555	CD1	ILE	A	90	26.784	-0.098	-10.613	1.00	35.17	6	C
ATOM	556	CG2	ILE	A	90	23.783	1.978	-11.861	1.00	29.24	6	C
ATOM	557	C	ILE	A	90	23.133	-0.383	-13.296	1.00	28.55	6	C
ATOM	558	O	ILE	A	90	23.617	-0.415	-14.422	1.00	28.28	8	O
ATOM	559	N	LEU	A	91	21.836	-0.227	-13.065	1.00	28.68	7	N
ATOM	560	CA	LEU	A	91	20.892	0.034	-14.139	1.00	29.39	6	C
ATOM	561	CB	LEU	A	91	19.790	-1.021	-14.138	1.00	29.32	6	C

ATOM	562	CG	LEU	A	91	18.627	-0.740	-15.096	1.00	30.30	6	C
ATOM	563	CD1	LEU	A	91	19.064	-0.984	-16.540	1.00	30.54	6	C
ATOM	564	CD2	LEU	A	91	17.408	-1.595	-14.738	1.00	30.62	6	C
ATOM	565	C	LEU	A	91	20.329	1.441	-13.885	1.00	29.83	6	C
ATOM	566	O	LEU	A	91	19.727	1.695	-12.830	1.00	29.50	8	O
ATOM	567	N	TYR	A	92	20.579	2.358	-14.821	1.00	30.23	7	N
ATOM	568	CA	TYR	A	92	20.127	3.751	-14.697	1.00	30.91	6	C
ATOM	569	CB	TYR	A	92	20.768	4.626	-15.772	1.00	30.86	6	C
ATOM	570	CG	TYR	A	92	22.249	4.831	-15.576	1.00	32.76	6	C
ATOM	571	CD1	TYR	A	92	23.140	3.777	-15.715	1.00	33.72	6	C
ATOM	572	CE1	TYR	A	92	24.493	3.958	-15.528	1.00	35.16	6	C
ATOM	573	CZ	TYR	A	92	24.973	5.204	-15.199	1.00	34.76	6	C
ATOM	574	OH	TYR	A	92	26.318	5.388	-15.021	1.00	35.17	8	O
ATOM	575	CE2	TYR	A	92	24.115	6.268	-15.056	1.00	34.84	6	C
ATOM	576	CD2	TYR	A	92	22.758	6.078	-15.238	1.00	34.70	6	C
ATOM	577	C	TYR	A	92	18.610	3.886	-14.743	1.00	31.31	6	C
ATOM	578	O	TYR	A	92	17.918	2.977	-15.190	1.00	31.18	8	O
ATOM	579	N	ASN	A	93	18.096	5.028	-14.283	1.00	32.12	7	N
ATOM	580	CA	ASN	A	93	16.650	5.235	-14.234	1.00	33.32	6	C
ATOM	581	CB	ASN	A	93	16.261	6.432	-13.344	1.00	33.25	6	C
ATOM	582	CG	ASN	A	93	16.786	7.768	-13.866	1.00	33.58	6	C
ATOM	583	OD1	ASN	A	93	17.268	7.874	-14.998	1.00	32.74	8	O
ATOM	584	ND2	ASN	A	93	16.697	8.802	-13.025	1.00	33.00	7	N
ATOM	585	C	ASN	A	93	15.951	5.319	-15.598	1.00	34.12	6	C
ATOM	586	O	ASN	A	93	14.728	5.469	-15.661	1.00	34.09	8	O
ATOM	587	N	ASP	A	94	16.708	5.244	-16.687	1.00	34.86	7	N
ATOM	588	CA	ASP	A	94	16.053	5.211	-17.998	1.00	35.81	6	C
ATOM	589	CB	ASP	A	94	16.846	5.942	-19.087	1.00	36.25	6	C
ATOM	590	CG	ASP	A	94	18.336	5.762	-18.951	1.00	38.64	6	C
ATOM	591	OD1	ASP	A	94	18.966	5.230	-19.897	1.00	36.80	8	O
ATOM	592	OD2	ASP	A	94	18.964	6.124	-17.927	1.00	44.80	8	O
ATOM	593	C	ASP	A	94	15.721	3.777	-18.384	1.00	35.67	6	C
ATOM	594	O	ASP	A	94	15.157	3.522	-19.453	1.00	35.81	8	O
ATOM	595	N	GLY	A	95	16.071	2.846	-17.498	1.00	35.30	7	N
ATOM	596	CA	GLY	A	95	15.730	1.441	-17.658	1.00	34.65	6	C
ATOM	597	C	GLY	A	95	16.512	0.649	-18.693	1.00	34.46	6	C
ATOM	598	O	GLY	A	95	16.176	-0.506	-18.967	1.00	34.40	8	O
ATOM	599	N	ASP	A	96	17.563	1.242	-19.250	1.00	34.02	7	N
ATOM	600	CA	ASP	A	96	18.339	0.568	-20.293	1.00	33.87	6	C
ATOM	601	CB	ASP	A	96	17.887	1.059	-21.675	1.00	33.75	6	C
ATOM	602	CG	ASP	A	96	18.427	0.203	-22.811	1.00	34.99	6	C
ATOM	603	OD1	ASP	A	96	18.663	-1.003	-22.608	1.00	34.90	8	O
ATOM	604	OD2	ASP	A	96	18.647	0.659	-23.949	1.00	36.72	8	O
ATOM	605	C	ASP	A	96	19.852	0.741	-20.129	1.00	33.04	6	C
ATOM	606	O	ASP	A	96	20.624	-0.175	-20.402	1.00	33.28	8	O
ATOM	607	N	SER	A	97	20.280	1.911	-19.671	1.00	32.38	7	N
ATOM	608	CA	SER	A	97	21.709	2.180	-19.519	1.00	31.14	6	C
ATOM	609	CB	SER	A	97	21.951	3.681	-19.327	1.00	31.84	6	C
ATOM	610	OG	SER	A	97	21.665	4.395	-20.523	1.00	31.58	8	O
ATOM	611	C	SER	A	97	22.330	1.387	-18.364	1.00	30.82	6	C
ATOM	612	O	SER	A	97	21.708	1.223	-17.307	1.00	29.82	8	O
ATOM	613	N	LEU	A	98	23.545	0.892	-18.575	1.00	29.55	7	N
ATOM	614	CA	LEU	A	98	24.223	0.093	-17.559	1.00	29.94	6	C
ATOM	615	CB	LEU	A	98	24.370	-1.359	-18.022	1.00	29.99	6	C
ATOM	616	CG	LEU	A	98	23.131	-2.222	-18.248	1.00	29.63	6	C
ATOM	617	CD1	LEU	A	98	23.554	-3.504	-18.966	1.00	30.60	6	C
ATOM	618	CD2	LEU	A	98	22.433	-2.544	-16.933	1.00	29.49	6	C

ATOM	619	C	LEU	A	98	25.612	0.614	-17.275	1.00	29.90	6	C
ATOM	620	O	LEU	A	98	26.267	1.186	-18.152	1.00	29.73	8	O
ATOM	621	N	GLN	A	99	26.055	0.397	-16.040	1.00	29.63	7	N
ATOM	622	CA	GLN	A	99	27.436	0.632	-15.656	1.00	29.72	6	C
ATOM	623	CB	GLN	A	99	27.580	1.834	-14.710	1.00	29.87	6	C
ATOM	624	CG	GLN	A	99	29.006	2.022	-14.179	1.00	31.65	6	C
ATOM	625	CD	GLN	A	99	29.154	3.179	-13.176	1.00	34.86	6	C
ATOM	626	OE1	GLN	A	99	28.225	3.975	-12.969	1.00	38.05	8	O
ATOM	627	NE2	GLN	A	99	30.320	3.268	-12.558	1.00	35.00	7	N
ATOM	628	C	GLN	A	99	27.878	-0.649	-14.948	1.00	29.09	6	C
ATOM	629	O	GLN	A	99	27.285	-1.027	-13.939	1.00	28.62	8	O
ATOM	630	N	TYR	A	100	28.885	-1.326	-15.496	1.00	28.74	7	N
ATOM	631	CA	TYR	A	100	29.428	-2.553	-14.902	1.00	28.36	6	C
ATOM	632	CB	TYR	A	100	29.588	-3.661	-15.952	1.00	28.26	6	C
ATOM	633	CG	TYR	A	100	29.947	-5.033	-15.395	1.00	28.17	6	C
ATOM	634	CD1	TYR	A	100	29.237	-5.587	-14.329	1.00	28.77	6	C
ATOM	635	CE1	TYR	A	100	29.544	-6.851	-13.835	1.00	28.30	6	C
ATOM	636	CZ	TYR	A	100	30.580	-7.566	-14.398	1.00	27.40	6	C
ATOM	637	OH	TYR	A	100	30.886	-8.818	-13.917	1.00	27.32	8	O
ATOM	638	CE2	TYR	A	100	31.303	-7.029	-15.446	1.00	27.53	6	C
ATOM	639	CD2	TYR	A	100	30.981	-5.782	-15.941	1.00	27.24	6	C
ATOM	640	C	TYR	A	100	30.782	-2.244	-14.300	1.00	28.71	6	C
ATOM	641	O	TYR	A	100	31.638	-1.639	-14.963	1.00	29.05	8	O
ATOM	642	N	ILE	A	101	30.973	-2.630	-13.040	1.00	28.17	7	N
ATOM	643	CA	ILE	A	101	32.246	-2.421	-12.363	1.00	28.15	6	C
ATOM	644	CB	ILE	A	101	32.094	-1.589	-11.065	1.00	28.44	6	C
ATOM	645	CG1	ILE	A	101	31.429	-0.235	-11.339	1.00	29.47	6	C
ATOM	646	CD1	ILE	A	101	29.939	-0.276	-11.252	1.00	32.69	6	C
ATOM	647	CG2	ILE	A	101	33.444	-1.322	-10.461	1.00	28.54	6	C
ATOM	648	C	ILE	A	101	32.816	-3.778	-12.014	1.00	28.30	6	C
ATOM	649	O	ILE	A	101	32.228	-4.518	-11.212	1.00	26.96	8	O
ATOM	650	N	GLU	A	102	33.951	-4.099	-12.625	1.00	28.36	7	N
ATOM	651	CA	GLU	A	102	34.618	-5.373	-12.391	1.00	29.48	6	C
ATOM	652	CB	GLU	A	102	35.545	-5.713	-13.558	1.00	29.40	6	C
ATOM	653	CG	GLU	A	102	34.783	-6.010	-14.839	1.00	29.63	6	C
ATOM	654	CD	GLU	A	102	35.686	-6.094	-16.049	1.00	31.22	6	C
ATOM	655	OE1	GLU	A	102	36.394	-7.111	-16.206	1.00	31.47	8	O
ATOM	656	OE2	GLU	A	102	35.691	-5.132	-16.841	1.00	34.12	8	O
ATOM	657	C	GLU	A	102	35.380	-5.349	-11.072	1.00	30.52	6	C
ATOM	658	O	GLU	A	102	35.519	-4.299	-10.446	1.00	29.92	8	O
ATOM	659	N	ARG	A	103	35.854	-6.518	-10.647	1.00	31.46	7	N
ATOM	660	CA	ARG	A	103	36.540	-6.644	-9.365	1.00	33.21	6	C
ATOM	661	CB	ARG	A	103	37.104	-8.056	-9.195	1.00	33.45	6	C
ATOM	662	CG	ARG	A	103	36.036	-9.120	-9.017	1.00	35.12	6	C
ATOM	663	CD	ARG	A	103	36.541	-10.534	-9.294	1.00	39.05	6	C
ATOM	664	NE	ARG	A	103	37.045	-11.209	-8.112	1.00	42.34	7	N
ATOM	665	CZ	ARG	A	103	38.106	-12.010	-8.100	1.00	43.44	6	C
ATOM	666	NH1	ARG	A	103	38.817	-12.210	-9.204	1.00	44.15	7	N
ATOM	667	NH2	ARG	A	103	38.474	-12.594	-6.972	1.00	44.98	7	N
ATOM	668	C	ARG	A	103	37.659	-5.641	-9.179	1.00	33.82	6	C
ATOM	669	O	ARG	A	103	37.835	-5.100	-8.101	1.00	34.04	8	O
ATOM	670	N	ASP	A	104	38.415	-5.400	-10.236	1.00	35.17	7	N
ATOM	671	CA	ASP	A	104	39.541	-4.483	-10.173	1.00	36.39	6	C
ATOM	672	CB	ASP	A	104	40.521	-4.855	-11.266	1.00	37.19	6	C
ATOM	673	CG	ASP	A	104	39.825	-5.106	-12.580	1.00	40.35	6	C
ATOM	674	OD1	ASP	A	104	39.635	-6.296	-12.946	1.00	45.85	8	O
ATOM	675	OD2	ASP	A	104	39.375	-4.177	-13.279	1.00	39.52	8	O

ATOM	676	C	ASP	A	104	39.114	-3.023	-10.344	1.00	36.08	6	C
ATOM	677	O	ASP	A	104	39.958	-2.139	-10.469	1.00	36.56	8	O
ATOM	678	N	GLY	A	105	37.812	-2.768	-10.359	1.00	35.42	7	N
ATOM	679	CA	GLY	A	105	37.328	-1.407	-10.502	1.00	35.09	6	C
ATOM	680	C	GLY	A	105	37.112	-0.922	-11.930	1.00	34.77	6	C
ATOM	681	O	GLY	A	105	36.659	0.201	-12.136	1.00	34.65	8	O
ATOM	682	N	THR	A	106	37.413	-1.754	-12.922	1.00	34.37	7	N
ATOM	683	CA	THR	A	106	37.198	-1.344	-14.315	1.00	34.21	6	C
ATOM	684	CB	THR	A	106	37.736	-2.400	-15.295	1.00	34.25	6	C
ATOM	685	OG1	THR	A	106	39.147	-2.568	-15.095	1.00	33.88	8	O
ATOM	686	CG2	THR	A	106	37.638	-1.888	-16.738	1.00	34.18	6	C
ATOM	687	C	THR	A	106	35.713	-1.081	-14.577	1.00	34.43	6	C
ATOM	688	O	THR	A	106	34.864	-1.919	-14.263	1.00	33.73	8	O
ATOM	689	N	GLU	A	107	35.401	0.080	-15.154	1.00	34.65	7	N
ATOM	690	CA	GLU	A	107	34.009	0.456	-15.410	1.00	35.83	6	C
ATOM	691	CB	GLU	A	107	33.721	1.882	-14.917	1.00	35.89	6	C
ATOM	692	CG	GLU	A	107	33.975	2.149	-13.440	1.00	38.03	6	C
ATOM	693	CD	GLU	A	107	33.784	3.616	-13.066	1.00	40.69	6	C
ATOM	694	OE1	GLU	A	107	33.359	4.413	-13.938	1.00	42.02	8	O
ATOM	695	OE2	GLU	A	107	34.062	3.982	-11.902	1.00	42.05	8	O
ATOM	696	C	GLU	A	107	33.649	0.369	-16.890	1.00	35.91	6	C
ATOM	697	O	GLU	A	107	34.394	0.848	-17.750	1.00	36.37	8	O
ATOM	698	N	SER	A	108	32.508	-0.244	-17.182	1.00	35.87	7	N
ATOM	699	CA	SER	A	108	32.015	-0.356	-18.551	1.00	36.36	6	C
ATOM	700	CB	SER	A	108	31.931	-1.820	-18.984	1.00	36.01	6	C
ATOM	701	OG	SER	A	108	33.217	-2.393	-19.154	1.00	36.34	8	O
ATOM	702	C	SER	A	108	30.624	0.261	-18.612	1.00	36.72	6	C
ATOM	703	O	SER	A	108	29.822	0.043	-17.708	1.00	36.15	8	O
ATOM	704	N	TYR	A	109	30.353	1.044	-19.658	1.00	36.80	7	N
ATOM	705	CA	TYR	A	109	29.036	1.654	-19.838	1.00	37.56	6	C
ATOM	706	CB	TYR	A	109	29.135	3.179	-19.970	1.00	37.52	6	C
ATOM	707	CG	TYR	A	109	29.685	3.801	-18.705	1.00	38.15	6	C
ATOM	708	CD1	TYR	A	109	31.044	3.771	-18.435	1.00	39.00	6	C
ATOM	709	CE1	TYR	A	109	31.560	4.308	-17.266	1.00	38.75	6	C
ATOM	710	CZ	TYR	A	109	30.710	4.878	-16.347	1.00	39.50	6	C
ATOM	711	OH	TYR	A	109	31.236	5.409	-15.186	1.00	39.40	8	O
ATOM	712	CE2	TYR	A	109	29.346	4.909	-16.586	1.00	39.18	6	C
ATOM	713	CD2	TYR	A	109	28.842	4.366	-17.759	1.00	38.80	6	C
ATOM	714	C	TYR	A	109	28.365	1.011	-21.036	1.00	37.99	6	C
ATOM	715	O	TYR	A	109	28.862	1.095	-22.159	1.00	38.19	8	O
ATOM	716	N	LEU	A	110	27.251	0.338	-20.777	1.00	38.47	7	N
ATOM	717	CA	LEU	A	110	26.557	-0.425	-21.803	1.00	39.08	6	C
ATOM	718	CB	LEU	A	110	26.799	-1.926	-21.594	1.00	39.57	6	C
ATOM	719	CG	LEU	A	110	28.213	-2.444	-21.365	1.00	40.82	6	C
ATOM	720	CD1	LEU	A	110	28.195	-3.950	-21.138	1.00	41.92	6	C
ATOM	721	CD2	LEU	A	110	29.094	-2.098	-22.558	1.00	42.45	6	C
ATOM	722	C	LEU	A	110	25.067	-0.202	-21.738	1.00	38.83	6	C
ATOM	723	O	LEU	A	110	24.565	0.584	-20.932	1.00	38.56	8	O
ATOM	724	N	THR	A	111	24.358	-0.912	-22.605	1.00	38.56	7	N
ATOM	725	CA	THR	A	111	22.911	-0.869	-22.607	1.00	38.47	6	C
ATOM	726	CB	THR	A	111	22.403	-0.170	-23.884	1.00	38.99	6	C
ATOM	727	OG1	THR	A	111	22.813	1.205	-23.879	1.00	40.54	8	O
ATOM	728	CG2	THR	A	111	20.922	-0.042	-23.840	1.00	40.63	6	C
ATOM	729	C	THR	A	111	22.389	-2.300	-22.523	1.00	37.37	6	C
ATOM	730	O	THR	A	111	23.043	-3.234	-23.004	1.00	37.13	8	O
ATOM	731	N	VAL	A	112	21.225	-2.480	-21.908	1.00	36.49	7	N
ATOM	732	CA	VAL	A	112	20.606	-3.797	-21.842	1.00	36.02	6	C

ATOM	733	CB	VAL	A	112	19.387	-3.814	-20.899	1.00	36.12	6	C
ATOM	734	CG1	VAL	A	112	18.649	-5.153	-20.973	1.00	35.72	6	C
ATOM	735	CG2	VAL	A	112	19.819	-3.524	-19.464	1.00	35.10	6	C
ATOM	736	C	VAL	A	112	20.179	-4.189	-23.260	1.00	36.45	6	C
ATOM	737	O	VAL	A	112	20.398	-5.317	-23.696	1.00	35.73	8	O
ATOM	738	N	SER	A	113	19.607	-3.229	-23.980	1.00	36.64	7	N
ATOM	739	CA	SER	A	113	19.122	-3.462	-25.338	1.00	37.54	6	C
ATOM	740	CB	SER	A	113	18.484	-2.190	-25.912	1.00	37.50	6	C
ATOM	741	OG	SER	A	113	19.411	-1.120	-25.988	1.00	38.07	8	O
ATOM	742	C	SER	A	113	20.203	-3.999	-26.273	1.00	37.96	6	C
ATOM	743	O	SER	A	113	19.897	-4.727	-27.224	1.00	38.50	8	O
ATOM	744	N	SER	A	114	21.459	-3.648	-26.005	1.00	38.01	7	N
ATOM	745	CA	SER	A	114	22.583	-4.107	-26.826	1.00	38.47	6	C
ATOM	746	CB	SER	A	114	23.839	-3.268	-26.562	1.00	38.34	6	C
ATOM	747	OG	SER	A	114	24.459	-3.627	-25.338	1.00	38.19	8	O
ATOM	748	C	SER	A	114	22.910	-5.588	-26.628	1.00	38.68	6	C
ATOM	749	O	SER	A	114	23.719	-6.155	-27.370	1.00	38.77	8	O
ATOM	750	N	HIS	A	115	22.296	-6.199	-25.619	1.00	38.57	7	N
ATOM	751	CA	HIS	A	115	22.502	-7.616	-25.308	1.00	38.72	6	C
ATOM	752	CB	HIS	A	115	21.892	-8.516	-26.398	1.00	39.09	6	C
ATOM	753	CG	HIS	A	115	21.849	-9.966	-26.026	1.00	39.56	6	C
ATOM	754	ND1	HIS	A	115	20.675	-10.623	-25.724	1.00	40.68	7	N
ATOM	755	CE1	HIS	A	115	20.941	-11.882	-25.428	1.00	40.16	6	C
ATOM	756	NE2	HIS	A	115	22.246	-12.066	-25.525	1.00	40.59	7	N
ATOM	757	CD2	HIS	A	115	22.836	-10.884	-25.899	1.00	39.60	6	C
ATOM	758	C	HIS	A	115	23.969	-7.997	-25.067	1.00	38.66	6	C
ATOM	759	O	HIS	A	115	24.575	-8.716	-25.871	1.00	38.76	8	O
ATOM	760	N	PRO	A	116	24.538	-7.509	-23.969	1.00	38.46	7	N
ATOM	761	CA	PRO	A	116	25.911	-7.855	-23.578	1.00	38.23	6	C
ATOM	762	CB	PRO	A	116	26.177	-6.905	-22.412	1.00	38.56	6	C
ATOM	763	CG	PRO	A	116	24.818	-6.660	-21.847	1.00	38.47	6	C
ATOM	764	CD	PRO	A	116	23.924	-6.544	-23.039	1.00	38.20	6	C
ATOM	765	C	PRO	A	116	25.963	-9.307	-23.103	1.00	37.95	6	C
ATOM	766	O	PRO	A	116	25.567	-9.620	-21.977	1.00	37.64	8	O
ATOM	767	N	ASN	A	117	26.460	-10.188	-23.966	1.00	37.62	7	N
ATOM	768	CA	ASN	A	117	26.448	-11.631	-23.712	1.00	37.09	6	C
ATOM	769	CB	ASN	A	117	27.238	-12.351	-24.816	1.00	37.51	6	C
ATOM	770	CG	ASN	A	117	26.686	-12.059	-26.199	1.00	38.58	6	C
ATOM	771	OD1	ASN	A	117	25.471	-12.017	-26.389	1.00	40.21	8	O
ATOM	772	ND2	ASN	A	117	27.571	-11.839	-27.169	1.00	40.37	7	N
ATOM	773	C	ASN	A	117	26.895	-12.111	-22.320	1.00	36.55	6	C
ATOM	774	O	ASN	A	117	26.206	-12.907	-21.671	1.00	36.33	8	O
ATOM	775	N	ALA	A	118	28.046	-11.630	-21.865	1.00	35.80	7	N
ATOM	776	CA	ALA	A	118	28.604	-12.066	-20.591	1.00	35.11	6	C
ATOM	777	CB	ALA	A	118	30.063	-11.596	-20.465	1.00	35.22	6	C
ATOM	778	C	ALA	A	118	27.786	-11.594	-19.385	1.00	34.02	6	C
ATOM	779	O	ALA	A	118	27.867	-12.181	-18.303	1.00	33.55	8	O
ATOM	780	N	LEU	A	119	26.972	-10.565	-19.587	1.00	33.40	7	N
ATOM	781	CA	LEU	A	119	26.227	-9.942	-18.491	1.00	33.05	6	C
ATOM	782	CB	LEU	A	119	26.328	-8.416	-18.609	1.00	33.25	6	C
ATOM	783	CG	LEU	A	119	27.690	-7.786	-18.323	1.00	34.22	6	C
ATOM	784	CD1	LEU	A	119	27.621	-6.275	-18.502	1.00	35.25	6	C
ATOM	785	CD2	LEU	A	119	28.119	-8.137	-16.917	1.00	36.37	6	C
ATOM	786	C	LEU	A	119	24.745	-10.322	-18.391	1.00	32.71	6	C
ATOM	787	O	LEU	A	119	24.071	-9.939	-17.436	1.00	31.84	8	O
ATOM	788	N	MET	A	120	24.234	-11.080	-19.356	1.00	32.22	7	N
ATOM	789	CA	MET	A	120	22.806	-11.400	-19.362	1.00	31.92	6	C

ATOM	790	CB	MET	A	120	22.444	-12.265	-20.567	1.00	32.59	6	C
ATOM	791	CG	MET	A	120	22.717	-11.571	-21.905	1.00	34.09	6	C
ATOM	792	SD	MET	A	120	22.013	-9.907	-22.079	1.00	38.77	16	S
ATOM	793	CE	MET	A	120	20.285	-10.247	-21.682	1.00	38.80	6	C
ATOM	794	C	MET	A	120	22.259	-11.979	-18.042	1.00	31.30	6	C
ATOM	795	O	MET	A	120	21.224	-11.531	-17.558	1.00	30.96	8	O
ATOM	796	N	LYS	A	121	22.960	-12.934	-17.441	1.00	30.40	7	N
ATOM	797	CA	LYS	A	121	22.510	-13.503	-16.167	1.00	30.26	6	C
ATOM	798	CB	LYS	A	121	23.373	-14.696	-15.761	1.00	30.27	6	C
ATOM	799	CG	LYS	A	121	23.150	-15.967	-16.604	1.00	33.46	6	C
ATOM	800	CD	LYS	A	121	23.970	-17.127	-16.066	1.00	35.55	6	C
ATOM	801	CE	LYS	A	121	23.753	-18.400	-16.889	1.00	38.65	6	C
ATOM	802	NZ	LYS	A	121	23.433	-18.087	-18.308	1.00	39.64	7	N
ATOM	803	C	LYS	A	121	22.513	-12.456	-15.025	1.00	29.34	6	C
ATOM	804	O	LYS	A	121	21.611	-12.425	-14.180	1.00	28.21	8	O
ATOM	805	N	LYS	A	122	23.540	-11.623	-14.989	1.00	28.61	7	N
ATOM	806	CA	LYS	A	122	23.610	-10.593	-13.951	1.00	28.77	6	C
ATOM	807	CB	LYS	A	122	25.008	-9.966	-13.893	1.00	28.40	6	C
ATOM	808	CG	LYS	A	122	26.040	-10.890	-13.202	1.00	28.80	6	C
ATOM	809	CD	LYS	A	122	27.469	-10.367	-13.341	1.00	27.89	6	C
ATOM	810	CE	LYS	A	122	28.417	-11.049	-12.351	1.00	29.28	6	C
ATOM	811	NZ	LYS	A	122	29.821	-10.547	-12.489	1.00	27.94	7	N
ATOM	812	C	LYS	A	122	22.502	-9.558	-14.155	1.00	28.77	6	C
ATOM	813	O	LYS	A	122	21.872	-9.110	-13.192	1.00	28.75	8	O
ATOM	814	N	ILE	A	123	22.247	-9.199	-15.408	1.00	28.79	7	N
ATOM	815	CA	ILE	A	123	21.157	-8.279	-15.725	1.00	29.61	6	C
ATOM	816	CB	ILE	A	123	21.107	-8.009	-17.237	1.00	29.77	6	C
ATOM	817	CG1	ILE	A	123	22.237	-7.059	-17.646	1.00	30.44	6	C
ATOM	818	CD1	ILE	A	123	22.525	-7.043	-19.147	1.00	30.78	6	C
ATOM	819	CG2	ILE	A	123	19.744	-7.442	-17.629	1.00	30.39	6	C
ATOM	820	C	ILE	A	123	19.807	-8.833	-15.256	1.00	29.86	6	C
ATOM	821	O	ILE	A	123	18.965	-8.097	-14.733	1.00	29.63	8	O
ATOM	822	N	THR	A	124	19.603	-10.135	-15.442	1.00	30.05	7	N
ATOM	823	CA	THR	A	124	18.347	-10.774	-15.045	1.00	30.32	6	C
ATOM	824	CB	THR	A	124	18.306	-12.235	-15.529	1.00	30.81	6	C
ATOM	825	OG1	THR	A	124	18.216	-12.258	-16.963	1.00	31.11	8	O
ATOM	826	CG2	THR	A	124	17.012	-12.909	-15.086	1.00	31.08	6	C
ATOM	827	C	THR	A	124	18.141	-10.705	-13.544	1.00	30.49	6	C
ATOM	828	O	THR	A	124	17.042	-10.417	-13.062	1.00	29.67	8	O
ATOM	829	N	LEU	A	125	19.212	-10.964	-12.807	1.00	30.74	7	N
ATOM	830	CA	LEU	A	125	19.172	-10.897	-11.356	1.00	31.48	6	C
ATOM	831	CB	LEU	A	125	20.505	-11.357	-10.780	1.00	31.93	6	C
ATOM	832	CG	LEU	A	125	20.591	-12.868	-10.563	1.00	34.56	6	C
ATOM	833	CD1	LEU	A	125	22.040	-13.324	-10.481	1.00	37.59	6	C
ATOM	834	CD2	LEU	A	125	19.841	-13.214	-9.287	1.00	36.58	6	C
ATOM	835	C	LEU	A	125	18.872	-9.475	-10.893	1.00	31.05	6	C
ATOM	836	O	LEU	A	125	18.117	-9.273	-9.945	1.00	31.71	8	O
ATOM	837	N	LEU	A	126	19.472	-8.494	-11.554	1.00	30.76	7	N
ATOM	838	CA	LEU	A	126	19.265	-7.098	-11.173	1.00	30.69	6	C
ATOM	839	CB	LEU	A	126	20.215	-6.172	-11.936	1.00	30.65	6	C
ATOM	840	CG	LEU	A	126	20.182	-4.691	-11.530	1.00	30.81	6	C
ATOM	841	CD1	LEU	A	126	20.007	-4.521	-10.014	1.00	31.78	6	C
ATOM	842	CD2	LEU	A	126	21.431	-3.961	-12.006	1.00	30.87	6	C
ATOM	843	C	LEU	A	126	17.815	-6.694	-11.397	1.00	31.15	6	C
ATOM	844	O	LEU	A	126	17.205	-6.038	-10.552	1.00	30.24	8	O
ATOM	845	N	LYS	A	127	17.256	-7.090	-12.538	1.00	31.51	7	N
ATOM	846	CA	LYS	A	127	15.858	-6.790	-12.814	1.00	32.55	6	C

ATOM	847	CB	LYS	A	127	15.478	-7.183	-14.238	1.00	32.83	6	C
ATOM	848	CG	LYS	A	127	15.983	-6.183	-15.249	1.00	35.21	6	C
ATOM	849	CD	LYS	A	127	15.786	-6.665	-16.663	1.00	38.59	6	C
ATOM	850	CE	LYS	A	127	16.504	-5.741	-17.630	1.00	40.76	6	C
ATOM	851	NZ	LYS	A	127	15.590	-4.779	-18.294	1.00	42.33	7	N
ATOM	852	C	LYS	A	127	14.944	-7.442	-11.786	1.00	32.75	6	C
ATOM	853	O	LYS	A	127	13.950	-6.842	-11.365	1.00	32.80	8	O
ATOM	854	N	TYR	A	128	15.268	-8.662	-11.372	1.00	33.10	7	N
ATOM	855	CA	TYR	A	128	14.495	-9.284	-10.298	1.00	33.78	6	C
ATOM	856	CB	TYR	A	128	15.046	-10.647	-9.904	1.00	34.29	6	C
ATOM	857	CG	TYR	A	128	14.322	-11.233	-8.714	1.00	37.18	6	C
ATOM	858	CD1	TYR	A	128	14.931	-11.307	-7.474	1.00	40.84	6	C
ATOM	859	CE1	TYR	A	128	14.270	-11.835	-6.382	1.00	42.52	6	C
ATOM	860	CZ	TYR	A	128	12.984	-12.297	-6.518	1.00	43.44	6	C
ATOM	861	OH	TYR	A	128	12.340	-12.821	-5.419	1.00	46.68	8	O
ATOM	862	CE2	TYR	A	128	12.347	-12.235	-7.736	1.00	42.86	6	C
ATOM	863	CD2	TYR	A	128	13.016	-11.700	-8.829	1.00	40.68	6	C
ATOM	864	C	TYR	A	128	14.497	-8.385	-9.059	1.00	33.36	6	C
ATOM	865	O	TYR	A	128	13.445	-8.136	-8.469	1.00	32.70	8	O
ATOM	866	N	PHE	A	129	15.683	-7.922	-8.659	1.00	33.07	7	N
ATOM	867	CA	PHE	A	129	15.797	-7.020	-7.501	1.00	33.01	6	C
ATOM	868	CB	PHE	A	129	17.255	-6.609	-7.244	1.00	33.14	6	C
ATOM	869	CG	PHE	A	129	18.074	-7.648	-6.523	1.00	34.24	6	C
ATOM	870	CD1	PHE	A	129	19.076	-8.325	-7.178	1.00	35.93	6	C
ATOM	871	CE1	PHE	A	129	19.839	-9.288	-6.523	1.00	36.77	6	C
ATOM	872	CZ	PHE	A	129	19.611	-9.552	-5.183	1.00	37.12	6	C
ATOM	873	CE2	PHE	A	129	18.624	-8.859	-4.505	1.00	36.61	6	C
ATOM	874	CD2	PHE	A	129	17.862	-7.914	-5.177	1.00	36.22	6	C
ATOM	875	C	PHE	A	129	14.956	-5.762	-7.707	1.00	32.81	6	C
ATOM	876	O	PHE	A	129	14.213	-5.347	-6.812	1.00	32.43	8	O
ATOM	877	N	ARG	A	130	15.090	-5.144	-8.876	1.00	32.83	7	N
ATOM	878	CA	ARG	A	130	14.347	-3.922	-9.173	1.00	33.47	6	C
ATOM	879	CB	ARG	A	130	14.625	-3.434	-10.595	1.00	33.52	6	C
ATOM	880	CG	ARG	A	130	13.696	-2.287	-11.010	1.00	34.77	6	C
ATOM	881	CD	ARG	A	130	13.624	-2.022	-12.500	1.00	36.82	6	C
ATOM	882	NE	ARG	A	130	13.117	-3.171	-13.245	1.00	37.03	7	N
ATOM	883	CZ	ARG	A	130	13.233	-3.298	-14.557	1.00	38.07	6	C
ATOM	884	NH1	ARG	A	130	13.833	-2.344	-15.253	1.00	39.49	7	N
ATOM	885	NH2	ARG	A	130	12.755	-4.370	-15.174	1.00	37.52	7	N
ATOM	886	C	ARG	A	130	12.845	-4.140	-9.000	1.00	33.31	6	C
ATOM	887	O	ARG	A	130	12.151	-3.328	-8.386	1.00	32.73	8	O
ATOM	888	N	ASN	A	131	12.352	-5.245	-9.548	1.00	33.24	7	N
ATOM	889	CA	ASN	A	131	10.933	-5.561	-9.480	1.00	33.80	6	C
ATOM	890	CB	ASN	A	131	10.596	-6.732	-10.406	1.00	33.96	6	C
ATOM	891	CG	ASN	A	131	10.819	-6.399	-11.867	1.00	35.72	6	C
ATOM	892	OD1	ASN	A	131	10.921	-5.229	-12.244	1.00	38.11	8	O
ATOM	893	ND2	ASN	A	131	10.893	-7.428	-12.702	1.00	36.73	7	N
ATOM	894	C	ASN	A	131	10.486	-5.870	-8.061	1.00	33.52	6	C
ATOM	895	O	ASN	A	131	9.419	-5.433	-7.640	1.00	33.38	8	O
ATOM	896	N	TYR	A	132	11.305	-6.617	-7.321	1.00	33.15	7	N
ATOM	897	CA	TYR	A	132	10.978	-6.933	-5.938	1.00	33.32	6	C
ATOM	898	CB	TYR	A	132	12.040	-7.850	-5.323	1.00	33.61	6	C
ATOM	899	CG	TYR	A	132	11.686	-8.294	-3.923	1.00	35.78	6	C
ATOM	900	CD1	TYR	A	132	11.066	-9.515	-3.700	1.00	36.19	6	C
ATOM	901	CE1	TYR	A	132	10.727	-9.920	-2.422	1.00	38.23	6	C
ATOM	902	CZ	TYR	A	132	11.009	-9.103	-1.352	1.00	38.11	6	C
ATOM	903	OH	TYR	A	132	10.674	-9.504	-0.077	1.00	39.25	8	O

ATOM	904	CE2	TYR	A	132	11.623	-7.886	-1.548	1.00	37.39	6	C
ATOM	905	CD2	TYR	A	132	11.958	-7.487	-2.825	1.00	36.22	6	C
ATOM	906	C	TYR	A	132	10.849	-5.658	-5.094	1.00	33.10	6	C
ATOM	907	O	TYR	A	132	9.887	-5.483	-4.336	1.00	32.61	8	O
ATOM	908	N	MET	A	133	11.821	-4.766	-5.236	1.00	32.43	7	N
ATOM	909	CA	MET	A	133	11.833	-3.533	-4.457	1.00	32.77	6	C
ATOM	910	CB	MET	A	133	13.163	-2.802	-4.644	1.00	32.05	6	C
ATOM	911	CG	MET	A	133	14.345	-3.557	-4.063	1.00	30.87	6	C
ATOM	912	SD	MET	A	133	15.894	-2.591	-4.095	1.00	29.32	16	S
ATOM	913	CE	MET	A	133	16.221	-2.588	-5.846	1.00	28.38	6	C
ATOM	914	C	MET	A	133	10.659	-2.621	-4.802	1.00	33.03	6	C
ATOM	915	O	MET	A	133	10.058	-2.009	-3.921	1.00	32.77	8	O
ATOM	916	N	SER	A	134	10.334	-2.538	-6.086	1.00	33.87	7	N
ATOM	917	CA	SER	A	134	9.218	-1.717	-6.533	1.00	35.17	6	C
ATOM	918	CB	SER	A	134	9.214	-1.615	-8.059	1.00	35.68	6	C
ATOM	919	OG	SER	A	134	7.952	-1.180	-8.534	1.00	37.31	8	O
ATOM	920	C	SER	A	134	7.867	-2.237	-6.021	1.00	35.37	6	C
ATOM	921	O	SER	A	134	6.973	-1.453	-5.694	1.00	35.81	8	O
ATOM	922	N	GLU	A	135	7.719	-3.553	-5.934	1.00	35.53	7	N
ATOM	923	CA	GLU	A	135	6.452	-4.133	-5.486	1.00	35.74	6	C
ATOM	924	CB	GLU	A	135	6.284	-5.545	-6.052	1.00	36.44	6	C
ATOM	925	CG	GLU	A	135	6.145	-5.599	-7.567	1.00	39.80	6	C
ATOM	926	CD	GLU	A	135	4.701	-5.532	-8.032	1.00	44.09	6	C
ATOM	927	OE1	GLU	A	135	3.819	-5.185	-7.213	1.00	45.82	8	O
ATOM	928	OE2	GLU	A	135	4.444	-5.840	-9.221	1.00	46.81	8	O
ATOM	929	C	GLU	A	135	6.251	-4.171	-3.967	1.00	34.96	6	C
ATOM	930	O	GLU	A	135	5.125	-4.011	-3.482	1.00	34.64	8	O
ATOM	931	N	HIS	A	136	7.333	-4.358	-3.216	1.00	33.49	7	N
ATOM	932	CA	HIS	A	136	7.220	-4.574	-1.777	1.00	32.95	6	C
ATOM	933	CB	HIS	A	136	7.933	-5.875	-1.405	1.00	33.31	6	C
ATOM	934	CG	HIS	A	136	7.430	-7.075	-2.142	1.00	35.42	6	C
ATOM	935	ND1	HIS	A	136	6.323	-7.787	-1.735	1.00	36.88	7	N
ATOM	936	CE1	HIS	A	136	6.118	-8.791	-2.570	1.00	38.14	6	C
ATOM	937	NE2	HIS	A	136	7.050	-8.752	-3.506	1.00	38.14	7	N
ATOM	938	CD2	HIS	A	136	7.884	-7.688	-3.261	1.00	37.14	6	C
ATOM	939	C	HIS	A	136	7.757	-3.503	-0.827	1.00	31.87	6	C
ATOM	940	O	HIS	A	136	7.369	-3.482	0.332	1.00	31.61	8	O
ATOM	941	N	LEU	A	137	8.643	-2.630	-1.297	1.00	30.88	7	N
ATOM	942	CA	LEU	A	137	9.362	-1.751	-0.361	1.00	30.11	6	C
ATOM	943	CB	LEU	A	137	10.871	-2.021	-0.444	1.00	29.63	6	C
ATOM	944	CG	LEU	A	137	11.300	-3.497	-0.339	1.00	29.18	6	C
ATOM	945	CD1	LEU	A	137	12.823	-3.621	-0.382	1.00	27.36	6	C
ATOM	946	CD2	LEU	A	137	10.755	-4.166	0.918	1.00	27.32	6	C
ATOM	947	C	LEU	A	137	9.108	-0.254	-0.468	1.00	29.92	6	C
ATOM	948	O	LEU	A	137	8.901	0.286	-1.545	1.00	29.22	8	O
ATOM	**949**	**N**	**LEU**	**A**	**138**	**9.165**	**0.401**	**0.687**	**1.00**	**29.80**	**7**	**N**
ATOM	**950**	**CA**	**LEU**	**A**	**138**	**8.936**	**1.834**	**0.809**	**1.00**	**30.37**	**6**	**C**
ATOM	**951**	**CB**	**LEU**	**A**	**138**	**8.575**	**2.142**	**2.255**	**1.00**	**30.84**	**6**	**C**
ATOM	**952**	**CG**	**LEU**	**A**	**138**	**8.219**	**3.594**	**2.534**	**1.00**	**31.89**	**6**	**C**
ATOM	**953**	**CD1**	**LEU**	**A**	**138**	**6.971**	**3.986**	**1.732**	**1.00**	**34.04**	**6**	**C**
ATOM	**954**	**CD2**	**LEU**	**A**	**138**	**7.996**	**3.742**	**4.020**	**1.00**	**35.04**	**6**	**C**
ATOM	**955**	**C**	**LEU**	**A**	**138**	**10.181**	**2.633**	**0.423**	**1.00**	**30.37**	**6**	**C**
ATOM	**956**	**O**	**LEU**	**A**	**138**	**11.286**	**2.272**	**0.805**	**1.00**	**30.44**	**8**	**O**
ATOM	957	N	LYS	A	139	10.008	3.703	-0.346	1.00	30.35	7	N
ATOM	958	CA	LYS	A	139	11.150	4.525	-0.761	1.00	30.71	6	C
ATOM	959	CB	LYS	A	139	10.823	5.264	-2.061	1.00	30.82	6	C
ATOM	960	CG	LYS	A	139	11.970	6.066	-2.618	1.00	31.83	6	C

ATOM	961	CD	LYS	A	139	11.733	6.531	-4.057	1.00	32.82	6	C
ATOM	962	CE	LYS	A	139	12.878	7.451	-4.495	1.00	32.63	6	C
ATOM	963	NZ	LYS	A	139	12.797	7.904	-5.908	1.00	32.89	7	N
ATOM	964	C	LYS	A	139	11.585	5.513	0.326	1.00	30.83	6	C
ATOM	965	O	LYS	A	139	10.830	6.420	0.692	1.00	30.70	8	O
ATOM	966	N	ALA	A	140	12.797	5.333	0.846	1.00	30.65	7	N
ATOM	967	CA	ALA	A	140	13.313	6.226	1.877	1.00	30.81	6	C
ATOM	968	CB	ALA	A	140	14.529	5.619	2.574	1.00	30.74	6	C
ATOM	969	C	ALA	A	140	13.667	7.586	1.288	1.00	31.03	6	C
ATOM	970	O	ALA	A	140	14.249	7.678	0.208	1.00	29.95	8	O
ATOM	971	N	GLY	A	141	13.302	8.642	2.010	1.00	31.87	7	N
ATOM	972	CA	GLY	A	141	13.600	9.989	1.568	1.00	33.39	6	C
ATOM	973	C	GLY	A	141	12.687	10.419	0.443	1.00	34.35	6	C
ATOM	974	O	GLY	A	141	13.056	11.249	-0.386	1.00	34.20	8	O
ATOM	975	N	ALA	A	142	11.490	9.852	0.421	1.00	35.76	7	N
ATOM	976	CA	ALA	A	142	10.509	10.176	-0.606	1.00	37.44	6	C
ATOM	977	CB	ALA	A	142	9.254	9.343	-0.410	1.00	37.52	6	C
ATOM	978	C	ALA	A	142	10.162	11.666	-0.588	1.00	38.51	6	C
ATOM	979	O	ALA	A	142	9.806	12.243	-1.618	1.00	38.73	8	O
ATOM	980	N	ASN	A	143	10.265	12.282	0.585	1.00	39.78	7	N
ATOM	981	CA	ASN	A	143	9.947	13.702	0.729	1.00	41.20	6	C
ATOM	982	CB	ASN	A	143	9.266	13.962	2.076	1.00	41.20	6	C
ATOM	983	CG	ASN	A	143	10.130	13.547	3.257	1.00	41.87	6	C
ATOM	984	OD1	ASN	A	143	11.169	12.898	3.087	1.00	42.25	8	O
ATOM	985	ND2	ASN	A	143	9.704	13.919	4.463	1.00	40.49	7	N
ATOM	986	C	ASN	A	143	11.163	14.611	0.581	1.00	42.02	6	C
ATOM	987	O	ASN	A	143	11.069	15.825	0.781	1.00	42.10	8	O
ATOM	988	N	ILE	A	144	12.307	14.031	0.230	1.00	42.79	7	N
ATOM	989	CA	ILE	A	144	13.530	14.817	0.099	1.00	43.76	6	C
ATOM	990	CB	ILE	A	144	14.733	14.093	0.739	1.00	43.56	6	C
ATOM	991	CG1	ILE	A	144	14.392	13.605	2.150	1.00	43.51	6	C
ATOM	992	CD1	ILE	A	144	15.560	12.895	2.865	1.00	44.14	6	C
ATOM	993	CG2	ILE	A	144	15.941	15.012	0.751	1.00	43.45	6	C
ATOM	994	C	ILE	A	144	13.858	15.124	-1.351	1.00	44.78	6	C
ATOM	995	O	ILE	A	144	13.718	14.266	-2.231	1.00	44.82	8	O
ATOM	996	N	THR	A	145	14.306	16.352	-1.588	1.00	46.00	7	N
ATOM	997	CA	THR	A	145	14.717	16.781	-2.914	1.00	47.32	6	C
ATOM	998	CB	THR	A	145	14.106	18.153	-3.251	1.00	47.48	6	C
ATOM	999	OG1	THR	A	145	12.681	18.032	-3.379	1.00	47.75	8	O
ATOM	1000	CG2	THR	A	145	14.555	18.603	-4.637	1.00	47.41	6	C
ATOM	1001	C	THR	A	145	16.236	16.870	-2.948	1.00	48.35	6	C
ATOM	1002	O	THR	A	145	16.824	17.752	-2.327	1.00	47.79	8	O
ATOM	1003	N	PRO	A	146	16.871	15.965	-3.687	1.00	49.56	7	N
ATOM	1004	CA	PRO	A	146	18.334	15.916	-3.748	1.00	50.70	6	C
ATOM	1005	CB	PRO	A	146	18.610	14.687	-4.623	1.00	50.57	6	C
ATOM	1006	CG	PRO	A	146	17.377	14.486	-5.410	1.00	50.15	6	C
ATOM	1007	CD	PRO	A	146	16.236	14.963	-4.561	1.00	49.76	6	C
ATOM	1008	C	PRO	A	146	18.870	17.157	-4.433	1.00	52.00	6	C
ATOM	1009	O	PRO	A	146	18.140	17.784	-5.203	1.00	52.03	8	O
ATOM	1010	N	ARG	A	147	20.114	17.525	-4.155	1.00	53.52	7	N
ATOM	1011	CA	ARG	A	147	20.694	18.665	-4.843	1.00	55.35	6	C
ATOM	1012	CB	ARG	A	147	21.863	19.266	-4.053	1.00	55.28	6	C
ATOM	1013	CG	ARG	A	147	23.214	18.590	-4.231	1.00	55.08	6	C
ATOM	1014	CD	ARG	A	147	24.306	19.208	-3.360	1.00	54.57	6	C
ATOM	1015	NE	ARG	A	147	25.650	18.751	-3.702	1.00	54.12	7	N
ATOM	1016	CZ	ARG	A	147	26.310	17.808	-3.044	1.00	54.07	6	C
ATOM	1017	NH1	ARG	A	147	25.750	17.199	-2.008	1.00	53.40	7	N

```
ATOM   1018  NH2 ARG A 147      27.533  17.464  -3.424  1.00 54.71      7       N
ATOM   1019  C   ARG A 147      21.114  18.207  -6.235  1.00 56.73      6       C
ATOM   1020  O   ARG A 147      20.917  17.047  -6.597  1.00 56.81      8       O
ATOM   1021  N   GLU A 148      21.667  19.114  -7.028  1.00 58.51      7       N
ATOM   1022  CA  GLU A 148      22.093  18.751  -8.371  1.00 60.30      6       C
ATOM   1023  CB  GLU A 148      21.094  19.278  -9.409  1.00 60.58      6       C
ATOM   1024  CG  GLU A 148      19.741  18.573  -9.355  1.00 61.94      6       C
ATOM   1025  CD  GLU A 148      18.802  18.971 -10.481  1.00 63.38      6       C
ATOM   1026  OE1 GLU A 148      19.203  19.779 -11.350  1.00 64.12      8       O
ATOM   1027  OE2 GLU A 148      17.655  18.470 -10.498  1.00 63.83      8       O
ATOM   1028  C   GLU A 148      23.503  19.255  -8.653  1.00 61.21      6       C
ATOM   1029  O   GLU A 148      23.875  20.351  -8.223  1.00 61.66      8       O
ATOM   1030  N   GLY A 149      24.286  18.446  -9.360  1.00 62.09      7       N
ATOM   1031  CA  GLY A 149      25.647  18.812  -9.718  1.00 62.88      6       C
ATOM   1032  C   GLY A 149      26.148  18.045 -10.929  1.00 63.42      6       C
ATOM   1033  O   GLY A 149      26.292  16.822 -10.881  1.00 63.58      8       O
ATOM   1034  N   ASP A 150      26.421  18.765 -12.015  1.00 63.96      7       N
ATOM   1035  CA  ASP A 150      26.880  18.152 -13.261  1.00 64.35      6       C
ATOM   1036  CB  ASP A 150      28.190  17.388 -13.049  1.00 64.61      6       C
ATOM   1037  CG  ASP A 150      29.268  18.247 -12.419  1.00 65.54      6       C
ATOM   1038  OD1 ASP A 150      29.231  19.484 -12.603  1.00 66.88      8       O
ATOM   1039  OD2 ASP A 150      30.189  17.775 -11.719  1.00 66.50      8       O
ATOM   1040  C   ASP A 150      25.809  17.226 -13.838  1.00 64.29      6       C
ATOM   1041  O   ASP A 150      24.663  17.639 -14.015  1.00 64.48      8       O
ATOM   1042  N   GLU A 151      26.192  15.982 -14.122  1.00 64.02      7       N
ATOM   1043  CA  GLU A 151      25.292  14.969 -14.684  1.00 63.67      6       C
ATOM   1044  CB  GLU A 151      24.029  15.603 -15.288  1.00 63.88      6       C
ATOM   1045  CG  GLU A 151      22.862  15.768 -14.322  1.00 64.91      6       C
ATOM   1046  CD  GLU A 151      21.638  16.391 -14.979  1.00 66.57      6       C
ATOM   1047  OE1 GLU A 151      21.428  16.155 -16.190  1.00 66.99      8       O
ATOM   1048  OE2 GLU A 151      20.882  17.113 -14.288  1.00 67.29      8       O
ATOM   1049  C   GLU A 151      26.001  14.137 -15.752  1.00 63.01      6       C
ATOM   1050  O   GLU A 151      25.558  13.040 -16.094  1.00 63.25      8       O
ATOM   1051  N   LEU A 152      27.110  14.662 -16.267  1.00 61.93      7       N
ATOM   1052  CA  LEU A 152      27.850  14.017 -17.354  1.00 60.75      6       C
ATOM   1053  CB  LEU A 152      28.805  15.023 -18.002  1.00 61.03      6       C
ATOM   1054  CG  LEU A 152      28.167  16.369 -18.359  1.00 61.49      6       C
ATOM   1055  CD1 LEU A 152      29.199  17.326 -18.939  1.00 62.25      6       C
ATOM   1056  CD2 LEU A 152      27.006  16.180 -19.326  1.00 62.22      6       C
ATOM   1057  C   LEU A 152      28.604  12.737 -16.961  1.00 59.54      6       C
ATOM   1058  O   LEU A 152      28.966  11.939 -17.826  1.00 59.82      8       O
ATOM   1059  N   ALA A 153      28.842  12.554 -15.664  1.00 57.73      7       N
ATOM   1060  CA  ALA A 153      29.528  11.370 -15.140  1.00 55.74      6       C
ATOM   1061  CB  ALA A 153      31.002  11.395 -15.533  1.00 55.94      6       C
ATOM   1062  C   ALA A 153      29.389  11.389 -13.624  1.00 54.05      6       C
ATOM   1063  O   ALA A 153      30.163  12.071 -12.951  1.00 54.29      8       O
ATOM   1064  N   ARG A 154      28.433  10.642 -13.065  1.00 51.55      7       N
ATOM   1065  CA  ARG A 154      28.196  10.803 -11.632  1.00 48.72      6       C
ATOM   1066  CB  ARG A 154      27.585  12.188 -11.413  1.00 48.98      6       C
ATOM   1067  CG  ARG A 154      26.314  12.428 -12.218  1.00 49.65      6       C
ATOM   1068  CD  ARG A 154      25.262  11.353 -12.036  1.00 50.74      6       C
ATOM   1069  NE  ARG A 154      23.946  11.781 -12.481  1.00 52.59      7       N
ATOM   1070  CZ  ARG A 154      22.901  10.977 -12.581  1.00 53.47      6       C
ATOM   1071  NH1 ARG A 154      23.016   9.695 -12.263  1.00 55.62      7       N
ATOM   1072  NH2 ARG A 154      21.737  11.452 -12.993  1.00 53.86      7       N
ATOM   1073  C   ARG A 154      27.355   9.811 -10.815  1.00 46.34      6       C
ATOM   1074  O   ARG A 154      26.956  10.148  -9.704  1.00 46.32      8       O
```

ATOM	1075	N	LEU	A	155	27.064	8.619	-11.318	1.00	42.78	7	N
ATOM	1076	CA	LEU	A	155	26.300	7.677	-10.496	1.00	39.40	6	C
ATOM	1077	CB	LEU	A	155	25.388	6.807	-11.360	1.00	39.89	6	C
ATOM	1078	CG	LEU	A	155	24.122	6.271	-10.690	1.00	40.23	6	C
ATOM	1079	CD1	LEU	A	155	23.327	7.409	-10.101	1.00	41.05	6	C
ATOM	1080	CD2	LEU	A	155	23.259	5.508	-11.679	1.00	40.15	6	C
ATOM	1081	C	LEU	A	155	27.262	6.804	-9.683	1.00	36.59	6	C
ATOM	1082	O	LEU	A	155	28.112	6.130	-10.249	1.00	35.75	8	O
ATOM	1083	N	PRO	A	156	27.140	6.825	-8.358	1.00	33.97	7	N
ATOM	1084	CA	PRO	A	156	28.030	6.030	-7.504	1.00	32.21	6	C
ATOM	1085	CB	PRO	A	156	27.895	6.711	-6.137	1.00	32.09	6	C
ATOM	1086	CG	PRO	A	156	26.505	7.245	-6.128	1.00	33.10	6	C
ATOM	1087	CD	PRO	A	156	26.175	7.614	-7.569	1.00	33.75	6	C
ATOM	1088	C	PRO	A	156	27.543	4.590	-7.382	1.00	30.42	6	C
ATOM	1089	O	PRO	A	156	26.371	4.322	-7.627	1.00	29.50	8	O
ATOM	1090	N	TYR	A	157	28.435	3.671	-7.017	1.00	28.48	7	N
ATOM	1091	CA	TYR	A	157	28.012	2.297	-6.793	1.00	27.62	6	C
ATOM	1092	CB	TYR	A	157	28.701	1.325	-7.750	1.00	27.64	6	C
ATOM	1093	CG	TYR	A	157	30.199	1.396	-7.711	1.00	28.30	6	C
ATOM	1094	CD1	TYR	A	157	30.927	0.684	-6.762	1.00	28.82	6	C
ATOM	1095	CE1	TYR	A	157	32.308	0.759	-6.724	1.00	30.62	6	C
ATOM	1096	CZ	TYR	A	157	32.966	1.538	-7.664	1.00	31.02	6	C
ATOM	1097	OH	TYR	A	157	34.336	1.621	-7.646	1.00	33.38	8	O
ATOM	1098	CE2	TYR	A	157	32.262	2.261	-8.600	1.00	29.83	6	C
ATOM	1099	CD2	TYR	A	157	30.895	2.184	-8.626	1.00	30.03	6	C
ATOM	1100	C	TYR	A	157	28.328	1.930	-5.354	1.00	26.56	6	C
ATOM	1101	O	TYR	A	157	29.028	2.657	-4.655	1.00	25.59	8	O
ATOM	1102	N	LEU	A	158	27.818	0.795	-4.917	1.00	26.50	7	N
ATOM	1103	CA	LEU	A	158	28.028	0.361	-3.549	1.00	25.95	6	C
ATOM	1104	CB	LEU	A	158	26.927	-0.627	-3.159	1.00	26.13	6	C
ATOM	1105	CG	LEU	A	158	26.975	-1.109	-1.717	1.00	24.82	6	C
ATOM	1106	CD1	LEU	A	158	26.752	0.064	-0.751	1.00	25.10	6	C
ATOM	1107	CD2	LEU	A	158	25.919	-2.197	-1.532	1.00	24.37	6	C
ATOM	1108	C	LEU	A	158	29.413	-0.286	-3.418	1.00	26.52	6	C
ATOM	1109	O	LEU	A	158	29.679	-1.318	-4.025	1.00	26.29	8	O
ATOM	1110	N	ARG	A	159	30.298	0.339	-2.648	1.00	26.73	7	N
ATOM	1111	CA	ARG	A	159	31.658	-0.176	-2.451	1.00	27.63	6	C
ATOM	1112	CB	ARG	A	159	32.561	0.902	-1.854	1.00	28.30	6	C
ATOM	1113	CG	ARG	A	159	33.108	1.914	-2.848	1.00	31.65	6	C
ATOM	1114	CD	ARG	A	159	34.205	2.815	-2.253	1.00	36.75	6	C
ATOM	1115	NE	ARG	A	159	34.803	3.710	-3.249	1.00	40.48	7	N
ATOM	1116	CZ	ARG	A	159	35.301	4.920	-2.979	1.00	42.21	6	C
ATOM	1117	NH1	ARG	A	159	35.282	5.406	-1.737	1.00	41.23	7	N
ATOM	1118	NH2	ARG	A	159	35.818	5.649	-3.960	1.00	44.20	7	N
ATOM	1119	C	ARG	A	159	31.672	-1.372	-1.513	1.00	27.88	6	C
ATOM	1120	O	ARG	A	159	32.331	-2.383	-1.771	1.00	27.60	8	O
ATOM	1121	N	THR	A	160	30.967	-1.234	-0.396	1.00	27.42	7	N
ATOM	1122	CA	THR	A	160	30.840	-2.330	0.557	1.00	28.18	6	C
ATOM	1123	CB	THR	A	160	32.181	-2.595	1.292	1.00	28.55	6	C
ATOM	1124	OG1	THR	A	160	32.102	-3.825	2.033	1.00	31.68	8	O
ATOM	1125	CG2	THR	A	160	32.441	-1.542	2.352	1.00	30.45	6	C
ATOM	1126	C	THR	A	160	29.721	-1.994	1.535	1.00	27.01	6	C
ATOM	1127	O	THR	A	160	29.190	-0.872	1.542	1.00	26.02	8	O
ATOM	1128	N	TRP	A	161	29.360	-2.974	2.345	1.00	26.52	7	N
ATOM	1129	CA	TRP	A	161	28.300	-2.817	3.325	1.00	25.88	6	C
ATOM	1130	CB	TRP	A	161	26.932	-2.972	2.657	1.00	25.89	6	C
ATOM	1131	CG	TRP	A	161	26.714	-4.349	2.082	1.00	26.34	6	C

ATOM	1132	CD1	TRP	A	161	27.141	-4.805	0.865	1.00	27.76	6	C
ATOM	1133	NE1	TRP	A	161	26.775	-6.119	0.694	1.00	27.89	7	N
ATOM	1134	CE2	TRP	A	161	26.093	-6.542	1.803	1.00	29.07	6	C
ATOM	1135	CD2	TRP	A	161	26.036	-5.453	2.702	1.00	28.34	6	C
ATOM	1136	CE3	TRP	A	161	25.390	-5.635	3.928	1.00	29.01	6	C
ATOM	1137	CZ3	TRP	A	161	24.820	-6.868	4.209	1.00	30.77	6	C
ATOM	1138	CH2	TRP	A	161	24.888	-7.928	3.292	1.00	30.47	6	C
ATOM	1139	CZ2	TRP	A	161	25.520	-7.787	2.088	1.00	30.10	6	C
ATOM	1140	C	TRP	A	161	28.462	-3.936	4.329	1.00	25.71	6	C
ATOM	1141	O	TRP	A	161	29.123	-4.933	4.046	1.00	25.32	8	O
ATOM	1142	N	PHE	A	162	27.851	-3.766	5.492	1.00	25.51	7	N
ATOM	1143	CA	PHE	A	162	27.813	-4.820	6.492	1.00	25.62	6	C
ATOM	1144	CB	PHE	A	162	29.146	-4.947	7.250	1.00	25.67	6	C
ATOM	1145	CG	PHE	A	162	29.507	-3.752	8.100	1.00	25.29	6	C
ATOM	1146	CD1	PHE	A	162	29.037	-3.645	9.408	1.00	25.53	6	C
ATOM	1147	CE1	PHE	A	162	29.370	-2.568	10.197	1.00	24.54	6	C
ATOM	1148	CZ	PHE	A	162	30.196	-1.566	9.693	1.00	25.18	6	C
ATOM	1149	CE2	PHE	A	162	30.673	-1.654	8.398	1.00	26.15	6	C
ATOM	1150	CD2	PHE	A	162	30.336	-2.758	7.610	1.00	26.22	6	C
ATOM	1151	C	PHE	A	162	26.622	-4.541	7.401	1.00	26.20	6	C
ATOM	1152	O	PHE	A	162	26.070	-3.432	7.386	1.00	25.37	8	O
ATOM	1153	N	ARG	A	163	26.202	-5.541	8.166	1.00	26.36	7	N
ATOM	1154	CA	ARG	A	163	25.066	-5.356	9.064	1.00	27.40	6	C
ATOM	1155	CB	ARG	A	163	23.899	-6.261	8.644	1.00	28.06	6	C
ATOM	1156	CG	ARG	A	163	24.291	-7.736	8.578	1.00	32.20	6	C
ATOM	1157	CD	ARG	A	163	23.123	-8.716	8.483	1.00	36.60	6	C
ATOM	1158	NE	ARG	A	163	22.269	-8.429	7.336	1.00	38.18	7	N
ATOM	1159	CZ	ARG	A	163	21.027	-7.987	7.435	1.00	39.16	6	C
ATOM	1160	NH1	ARG	A	163	20.488	-7.791	8.633	1.00	40.03	7	N
ATOM	1161	NH2	ARG	A	163	20.315	-7.755	6.343	1.00	39.07	7	N
ATOM	1162	C	ARG	A	163	25.463	-5.684	10.492	1.00	26.54	6	C
ATOM	1163	O	ARG	A	163	26.263	-6.594	10.717	1.00	26.51	8	O
ATOM	1164	N	THR	A	164	24.947	-4.906	11.442	1.00	26.05	7	N
ATOM	1165	CA	THR	A	164	25.115	-5.214	12.867	1.00	26.45	6	C
ATOM	1166	CB	THR	A	164	25.641	-4.012	13.660	1.00	26.07	6	C
ATOM	1167	OG1	THR	A	164	24.684	-2.945	13.600	1.00	25.53	8	O
ATOM	1168	CG2	THR	A	164	26.901	-3.434	13.016	1.00	26.06	6	C
ATOM	1169	C	THR	A	164	23.731	-5.605	13.385	1.00	26.76	6	C
ATOM	1170	O	THR	A	164	22.777	-5.676	12.613	1.00	26.66	8	O
ATOM	1171	N	ARG	A	165	23.602	-5.828	14.684	1.00	27.66	7	N
ATOM	1172	CA	ARG	A	165	22.295	-6.172	15.225	1.00	28.33	6	C
ATOM	1173	CB	ARG	A	165	22.429	-6.707	16.661	1.00	28.64	6	C
ATOM	1174	CG	ARG	A	165	22.973	-5.690	17.667	1.00	29.97	6	C
ATOM	1175	CD	ARG	A	165	23.038	-6.213	19.116	1.00	32.01	6	C
ATOM	1176	NE	ARG	A	165	23.350	-5.147	20.065	1.00	33.50	7	N
ATOM	1177	CZ	ARG	A	165	23.006	-5.159	21.346	1.00	34.29	6	C
ATOM	1178	NH1	ARG	A	165	22.335	-6.191	21.846	1.00	35.50	7	N
ATOM	1179	NH2	ARG	A	165	23.337	-4.140	22.132	1.00	35.43	7	N
ATOM	1180	C	ARG	A	165	21.381	-4.942	15.189	1.00	28.74	6	C
ATOM	1181	O	ARG	A	165	20.151	-5.064	15.256	1.00	29.22	8	O
ATOM	1182	N	SER	A	166	21.982	-3.759	15.061	1.00	27.82	7	N
ATOM	1183	CA	SER	A	166	21.228	-2.508	15.132	1.00	27.68	6	C
ATOM	1184	CB	SER	A	166	21.906	-1.526	16.103	1.00	27.82	6	C
ATOM	1185	OG	SER	A	166	22.192	-2.112	17.363	1.00	30.66	8	O
ATOM	1186	C	SER	A	166	21.036	-1.773	13.803	1.00	26.93	6	C
ATOM	1187	O	SER	A	166	20.139	-0.936	13.688	1.00	26.34	8	O
ATOM	1188	N	ALA	A	167	21.871	-2.066	12.811	1.00	25.82	7	N

ATOM	1189	CA	ALA	A	167	21.812	-1.282	11.584	1.00	25.43	6	C
ATOM	1190	CB	ALA	A	167	22.478	0.087	11.839	1.00	24.92	6	C
ATOM	1191	C	ALA	A	167	22.485	-1.926	10.390	1.00	24.77	6	C
ATOM	1192	O	ALA	A	167	23.257	-2.884	10.538	1.00	24.32	8	O
ATOM	1193	N	ILE	A	168	22.197	-1.371	9.210	1.00	24.07	7	N
ATOM	1194	CA	ILE	A	168	22.915	-1.733	7.997	1.00	24.20	6	C
ATOM	1195	CB	ILE	A	168	21.979	-2.205	6.840	1.00	24.66	6	C
ATOM	1196	CG1	ILE	A	168	22.816	-2.618	5.625	1.00	24.70	6	C
ATOM	1197	CD1	ILE	A	168	22.053	-3.476	4.600	1.00	26.32	6	C
ATOM	1198	CG2	ILE	A	168	20.959	-1.138	6.458	1.00	24.77	6	C
ATOM	1199	C	ILE	A	168	23.746	-0.507	7.618	1.00	23.55	6	C
ATOM	1200	O	ILE	A	168	23.278	0.636	7.720	1.00	23.26	8	O
ATOM	1201	N	ILE	A	169	25.000	-0.747	7.251	1.00	23.24	7	N
ATOM	1202	CA	ILE	A	169	25.950	0.326	6.933	1.00	22.80	6	C
ATOM	1203	CB	ILE	A	169	27.248	0.165	7.804	1.00	23.33	6	C
ATOM	1204	CG1	ILE	A	169	26.972	0.486	9.276	1.00	22.62	6	C
ATOM	1205	CD1	ILE	A	169	26.159	-0.586	10.034	1.00	23.51	6	C
ATOM	1206	CG2	ILE	A	169	28.389	1.042	7.284	1.00	22.78	6	C
ATOM	1207	C	ILE	A	169	26.269	0.178	5.461	1.00	22.89	6	C
ATOM	1208	O	ILE	A	169	26.661	-0.898	5.037	1.00	22.95	8	O
ATOM	1209	N	LEU	A	170	26.081	1.253	4.694	1.00	22.59	7	N
ATOM	1210	CA	LEU	A	170	26.267	1.254	3.253	1.00	23.18	6	C
ATOM	1211	CB	LEU	A	170	24.942	1.584	2.565	1.00	23.34	6	C
ATOM	1212	CG	LEU	A	170	23.794	0.623	2.903	1.00	23.78	6	C
ATOM	1213	CD1	LEU	A	170	22.445	1.335	2.810	1.00	24.60	6	C
ATOM	1214	CD2	LEU	A	170	23.847	-0.553	1.958	1.00	24.60	6	C
ATOM	1215	C	LEU	A	170	27.315	2.296	2.879	1.00	23.39	6	C
ATOM	1216	O	LEU	A	170	27.197	3.464	3.241	1.00	23.48	8	O
ATOM	1217	N	HIS	A	171	28.331	1.870	2.137	1.00	23.66	7	N
ATOM	1218	CA	HIS	A	171	29.408	2.766	1.751	1.00	24.20	6	C
ATOM	1219	CB	HIS	A	171	30.751	2.196	2.220	1.00	24.28	6	C
ATOM	1220	CG	HIS	A	171	31.930	3.061	1.885	1.00	26.53	6	C
ATOM	1221	ND1	HIS	A	171	31.832	4.424	1.718	1.00	27.97	7	N
ATOM	1222	CE1	HIS	A	171	33.029	4.922	1.446	1.00	29.66	6	C
ATOM	1223	NE2	HIS	A	171	33.901	3.931	1.443	1.00	31.07	7	N
ATOM	1224	CD2	HIS	A	171	33.239	2.755	1.712	1.00	27.93	6	C
ATOM	1225	C	HIS	A	171	29.408	2.911	0.238	1.00	24.50	6	C
ATOM	1226	O	HIS	A	171	29.715	1.950	-0.481	1.00	24.62	8	O
ATOM	1227	N	LEU	A	172	29.065	4.107	-0.229	1.00	24.25	7	N
ATOM	1228	CA	LEU	A	172	28.988	4.403	-1.660	1.00	25.47	6	C
ATOM	1229	CB	LEU	A	172	27.897	5.462	-1.915	1.00	25.00	6	C
ATOM	1230	CG	LEU	A	172	26.453	5.059	-1.582	1.00	27.92	6	C
ATOM	1231	CD1	LEU	A	172	25.466	6.107	-2.079	1.00	28.86	6	C
ATOM	1232	CD2	LEU	A	172	26.112	3.706	-2.191	1.00	28.96	6	C
ATOM	1233	C	LEU	A	172	30.340	4.863	-2.227	1.00	25.07	6	C
ATOM	1234	O	LEU	A	172	31.170	5.427	-1.499	1.00	24.85	8	O
ATOM	1235	N	SER	A	173	30.537	4.664	-3.528	1.00	25.35	7	N
ATOM	1236	CA	SER	A	173	31.809	5.003	-4.182	1.00	25.98	6	C
ATOM	1237	CB	SER	A	173	31.879	4.396	-5.590	1.00	26.00	6	C
ATOM	1238	OG	SER	A	173	30.838	4.902	-6.402	1.00	24.57	8	O
ATOM	1239	C	SER	A	173	32.102	6.507	-4.241	1.00	26.40	6	C
ATOM	1240	O	SER	A	173	33.217	6.912	-4.578	1.00	27.27	8	O
ATOM	1241	N	ASN	A	174	31.106	7.336	-3.938	1.00	26.37	7	N
ATOM	1242	CA	ASN	A	174	31.326	8.786	-3.902	1.00	26.18	6	C
ATOM	1243	CB	ASN	A	174	30.071	9.556	-4.355	1.00	26.32	6	C
ATOM	1244	CG	ASN	A	174	28.887	9.340	-3.444	1.00	26.00	6	C
ATOM	1245	OD1	ASN	A	174	28.971	8.617	-2.442	1.00	24.32	8	O

ATOM	1246	ND2	ASN	A	174	27.767	9.982	-3.778	1.00	26.41	7	N
ATOM	1247	C	ASN	A	174	31.806	9.248	-2.515	1.00	26.35	6	C
ATOM	1248	O	ASN	A	174	31.957	10.456	-2.262	1.00	26.26	8	O
ATOM	1249	N	GLY	A	175	32.054	8.275	-1.636	1.00	25.33	7	N
ATOM	1250	CA	GLY	A	175	32.550	8.526	-0.291	1.00	25.96	6	C
ATOM	1251	C	GLY	A	175	31.475	8.588	0.787	1.00	25.46	6	C
ATOM	1252	O	GLY	A	175	31.776	8.570	1.980	1.00	25.69	8	O
ATOM	1253	N	SER	A	176	30.216	8.675	0.374	1.00	25.33	7	N
ATOM	1254	CA	SER	A	176	29.115	8.757	1.335	1.00	25.17	6	C
ATOM	1255	CB	SER	A	176	27.797	9.051	0.608	1.00	25.53	6	C
ATOM	1256	OG	SER	A	176	27.809	10.376	0.083	1.00	28.20	8	O
ATOM	1257	C	SER	A	176	28.961	7.466	2.134	1.00	24.26	6	C
ATOM	1258	O	SER	A	176	29.149	6.373	1.600	1.00	23.81	8	O
ATOM	1259	N	VAL	A	177	28.617	7.608	3.411	1.00	23.56	7	N
ATOM	1260	CA	VAL	A	177	28.325	6.464	4.267	1.00	23.31	6	C
ATOM	1261	CB	VAL	A	177	29.328	6.306	5.428	1.00	23.77	6	C
ATOM	1262	CG1	VAL	A	177	28.852	5.217	6.392	1.00	24.27	6	C
ATOM	1263	CG2	VAL	A	177	30.722	5.959	4.891	1.00	23.70	6	C
ATOM	1264	C	VAL	A	177	26.907	6.634	4.813	1.00	23.11	6	C
ATOM	1265	O	VAL	A	177	26.578	7.683	5.388	1.00	22.34	8	O
ATOM	1266	N	GLN	A	178	26.067	5.619	4.603	1.00	22.44	7	N
ATOM	1267	CA	GLN	A	178	24.684	5.670	5.074	1.00	22.30	6	C
ATOM	1268	CB	GLN	A	178	23.712	5.489	3.901	1.00	22.62	6	C
ATOM	1269	CG	GLN	A	178	22.230	5.506	4.290	1.00	23.11	6	C
ATOM	1270	CD	GLN	A	178	21.321	5.526	3.078	1.00	24.18	6	C
ATOM	1271	OE1	GLN	A	178	21.713	5.999	2.004	1.00	24.06	8	O
ATOM	1272	NE2	GLN	A	178	20.114	4.996	3.235	1.00	23.29	7	N
ATOM	1273	C	GLN	A	178	24.459	4.594	6.122	1.00	21.55	6	C
ATOM	1274	O	GLN	A	178	24.934	3.473	5.977	1.00	21.29	8	O
ATOM	1275	N	ILE	A	179	23.776	4.955	7.202	1.00	21.05	7	N
ATOM	1276	CA	ILE	A	179	23.473	3.995	8.264	1.00	21.59	6	C
ATOM	1277	CB	ILE	A	179	24.273	4.340	9.570	1.00	21.66	6	C
ATOM	1278	CG1	ILE	A	179	25.773	4.433	9.280	1.00	21.63	6	C
ATOM	1279	CD1	ILE	A	179	26.620	4.908	10.469	1.00	22.78	6	C
ATOM	1280	CG2	ILE	A	179	24.021	3.268	10.644	1.00	22.47	6	C
ATOM	1281	C	ILE	A	179	21.976	4.045	8.532	1.00	21.86	6	C
ATOM	1282	O	ILE	A	179	21.451	5.108	8.902	1.00	21.55	8	O
ATOM	1283	N	ASN	A	180	21.304	2.910	8.335	1.00	22.26	7	N
ATOM	1284	CA	ASN	A	180	19.870	2.769	8.567	1.00	23.11	6	C
ATOM	1285	CB	ASN	A	180	19.183	2.006	7.422	1.00	22.91	6	C
ATOM	1286	CG	ASN	A	180	19.032	2.825	6.152	1.00	23.65	6	C
ATOM	1287	OD1	ASN	A	180	19.694	3.852	5.955	1.00	23.20	8	O
ATOM	1288	ND2	ASN	A	180	18.151	2.362	5.267	1.00	24.48	7	N
ATOM	1289	C	ASN	A	180	19.679	1.948	9.823	1.00	23.44	6	C
ATOM	1290	O	ASN	A	180	20.101	0.791	9.868	1.00	23.66	8	O
ATOM	1291	N	PHE	A	181	19.049	2.537	10.837	1.00	24.40	7	N
ATOM	1292	CA	PHE	A	181	18.801	1.842	12.097	1.00	25.35	6	C
ATOM	1293	CB	PHE	A	181	18.794	2.827	13.277	1.00	24.90	6	C
ATOM	1294	CG	PHE	A	181	20.128	3.511	13.500	1.00	25.75	6	C
ATOM	1295	CD1	PHE	A	181	20.368	4.774	12.996	1.00	25.28	6	C
ATOM	1296	CE1	PHE	A	181	21.604	5.392	13.186	1.00	26.59	6	C
ATOM	1297	CZ	PHE	A	181	22.597	4.744	13.886	1.00	26.44	6	C
ATOM	1298	CE2	PHE	A	181	22.372	3.494	14.388	1.00	27.49	6	C
ATOM	1299	CD2	PHE	A	181	21.139	2.873	14.190	1.00	25.89	6	C
ATOM	1300	C	PHE	A	181	17.498	1.049	12.007	1.00	26.42	6	C
ATOM	1301	O	PHE	A	181	16.460	1.585	11.601	1.00	26.61	8	O
ATOM	1302	N	PHE	A	182	17.564	-0.229	12.369	1.00	27.90	7	N

ATOM	1303	CA	PHE	A	182	16.421	-1.137	12.216	1.00	29.59	6	C
ATOM	1304	CB	PHE	A	182	16.851	-2.602	12.398	1.00	29.28	6	C
ATOM	1305	CG	PHE	A	182	17.902	-3.069	11.423	1.00	28.88	6	C
ATOM	1306	CD1	PHE	A	182	19.009	-3.762	11.875	1.00	28.98	6	C
ATOM	1307	CE1	PHE	A	182	19.982	-4.199	10.992	1.00	29.40	6	C
ATOM	1308	CZ	PHE	A	182	19.848	-3.955	9.638	1.00	29.35	6	C
ATOM	1309	CE2	PHE	A	182	18.743	-3.263	9.172	1.00	29.15	6	C
ATOM	1310	CD2	PHE	A	182	17.776	-2.831	10.067	1.00	29.92	6	C
ATOM	1311	C	PHE	A	182	15.264	-0.852	13.173	1.00	30.82	6	C
ATOM	1312	O	PHE	A	182	14.102	-0.862	12.772	1.00	32.18	8	O
ATOM	1313	N	GLN	A	183	15.591	-0.583	14.428	1.00	32.18	7	N
ATOM	1314	CA	GLN	A	183	14.591	-0.443	15.491	1.00	33.29	6	C
ATOM	1315	CB	GLN	A	183	15.304	-0.334	16.839	1.00	33.98	6	C
ATOM	1316	CG	GLN	A	183	14.415	-0.518	18.052	1.00	37.89	6	C
ATOM	1317	CD	GLN	A	183	15.216	-0.852	19.295	1.00	41.93	6	C
ATOM	1318	OE1	GLN	A	183	16.179	-0.149	19.629	1.00	44.34	8	O
ATOM	1319	NE2	GLN	A	183	14.829	-1.921	19.982	1.00	43.28	7	N
ATOM	1320	C	GLN	A	183	13.601	0.713	15.328	1.00	32.74	6	C
ATOM	1321	O	GLN	A	183	12.392	0.534	15.504	1.00	33.69	8	O
ATOM	1322	N	ASP	A	184	14.093	1.891	14.973	1.00	31.46	7	N
ATOM	1323	CA	ASP	A	184	13.210	3.048	14.889	1.00	30.53	6	C
ATOM	1324	CB	ASP	A	184	13.603	4.079	15.944	1.00	30.82	6	C
ATOM	1325	CG	ASP	A	184	15.022	4.596	15.763	1.00	31.38	6	C
ATOM	1326	OD1	ASP	A	184	15.690	4.271	14.741	1.00	30.84	8	O
ATOM	1327	OD2	ASP	A	184	15.547	5.350	16.601	1.00	31.85	8	O
ATOM	1328	C	ASP	A	184	13.142	3.688	13.510	1.00	29.56	6	C
ATOM	1329	O	ASP	A	184	12.552	4.752	13.340	1.00	28.90	8	O
ATOM	**1330**	**N**	**HIS**	**A**	**185**	**13.761**	**3.036**	**12.527**	**1.00**	**28.47**	**7**	**N**
ATOM	**1331**	**CA**	**HIS**	**A**	**185**	**13.753**	**3.525**	**11.155**	**1.00**	**28.01**	**6**	**C**
ATOM	**1332**	**CB**	**HIS**	**A**	**185**	**12.316**	**3.630**	**10.643**	**1.00**	**28.20**	**6**	**C**
ATOM	**1333**	**CG**	**HIS**	**A**	**185**	**11.530**	**2.365**	**10.792**	**1.00**	**30.16**	**6**	**C**
ATOM	**1334**	**ND1**	**HIS**	**A**	**185**	**11.815**	**1.223**	**10.075**	**1.00**	**30.90**	**7**	**N**
ATOM	**1335**	**CE1**	**HIS**	**A**	**185**	**10.958**	**0.271**	**10.405**	**1.00**	**33.28**	**6**	**C**
ATOM	**1336**	**NE2**	**HIS**	**A**	**185**	**10.126**	**0.756**	**11.310**	**1.00**	**32.23**	**7**	**N**
ATOM	**1337**	**CD2**	**HIS**	**A**	**185**	**10.464**	**2.063**	**11.574**	**1.00**	**32.17**	**6**	**C**
ATOM	**1338**	**C**	**HIS**	**A**	**185**	**14.480**	**4.858**	**10.936**	**1.00**	**26.92**	**6**	**C**
ATOM	**1339**	**O**	**HIS**	**A**	**185**	**14.302**	**5.486**	**9.886**	**1.00**	**26.86**	**8**	**O**
ATOM	1340	N	THR	A	186	15.279	5.303	11.905	1.00	26.53	7	N
ATOM	1341	CA	THR	A	186	16.040	6.552	11.714	1.00	25.66	6	C
ATOM	1342	CB	THR	A	186	16.544	7.190	13.041	1.00	25.96	6	C
ATOM	1343	OG1	THR	A	186	17.269	6.228	13.806	1.00	25.64	8	O
ATOM	1344	CG2	THR	A	186	15.389	7.622	13.962	1.00	26.85	6	C
ATOM	1345	C	THR	A	186	17.237	6.255	10.810	1.00	25.19	6	C
ATOM	1346	O	THR	A	186	17.713	5.121	10.769	1.00	24.35	8	O
ATOM	**1347**	**N**	**LYS**	**A**	**187**	**17.753**	**7.278**	**10.133**	**1.00**	**24.27**	**7**	**N**
ATOM	**1348**	**CA**	**LYS**	**A**	**187**	**18.831**	**7.065**	**9.158**	**1.00**	**24.03**	**6**	**C**
ATOM	**1349**	**CB**	**LYS**	**A**	**187**	**18.240**	**6.886**	**7.748**	**1.00**	**24.02**	**6**	**C**
ATOM	**1350**	**CG**	**LYS**	**A**	**187**	**17.219**	**5.759**	**7.624**	**1.00**	**23.58**	**6**	**C**
ATOM	**1351**	**CD**	**LYS**	**A**	**187**	**16.588**	**5.693**	**6.221**	**1.00**	**25.16**	**6**	**C**
ATOM	**1352**	**CE**	**LYS**	**A**	**187**	**15.579**	**4.537**	**6.132**	**1.00**	**24.31**	**6**	**C**
ATOM	**1353**	**NZ**	**LYS**	**A**	**187**	**14.412**	**4.734**	**7.055**	**1.00**	**24.25**	**7**	**N**
ATOM	**1354**	**C**	**LYS**	**A**	**187**	**19.776**	**8.251**	**9.120**	**1.00**	**24.10**	**6**	**C**
ATOM	**1355**	**O**	**LYS**	**A**	**187**	**19.354**	**9.391**	**9.325**	**1.00**	**24.85**	**8**	**O**
ATOM	1356	N	LEU	A	188	21.047	7.975	8.851	1.00	23.49	7	N
ATOM	1357	CA	LEU	A	188	22.055	9.014	8.674	1.00	23.57	6	C
ATOM	1358	CB	LEU	A	188	23.187	8.823	9.673	1.00	24.22	6	C
ATOM	1359	CG	LEU	A	188	22.913	8.997	11.160	1.00	25.94	6	C

ATOM	1360	CD1	LEU	A	188	24.207	8.835	11.924	1.00	26.57	6	C
ATOM	1361	CD2	LEU	A	188	22.320	10.366	11.419	1.00	28.31	6	C
ATOM	1362	C	LEU	A	188	22.677	8.861	7.297	1.00	23.47	6	C
ATOM	1363	O	LEU	A	188	22.961	7.735	6.877	1.00	22.53	8	O
ATOM	1364	N	ILE	A	189	22.901	9.978	6.605	1.00	23.57	7	N
ATOM	1365	CA	ILE	A	189	23.648	9.965	5.348	1.00	24.10	6	C
ATOM	1366	CB	ILE	A	189	22.783	10.443	4.181	1.00	24.59	6	C
ATOM	1367	CG1	ILE	A	189	21.523	9.575	4.044	1.00	24.75	6	C
ATOM	1368	CD1	ILE	A	189	20.426	10.284	3.221	1.00	25.98	6	C
ATOM	1369	CG2	ILE	A	189	23.592	10.403	2.890	1.00	25.55	6	C
ATOM	1370	C	ILE	A	189	24.822	10.929	5.555	1.00	24.28	6	C
ATOM	1371	O	ILE	A	189	24.613	12.129	5.705	1.00	23.81	8	O
ATOM	1372	N	LEU	A	190	26.034	10.383	5.611	1.00	24.25	7	N
ATOM	1373	CA	LEU	A	190	27.240	11.151	5.873	1.00	25.06	6	C
ATOM	1374	CB	LEU	A	190	28.137	10.388	6.850	1.00	25.79	6	C
ATOM	1375	CG	LEU	A	190	27.580	10.239	8.263	1.00	27.20	6	C
ATOM	1376	CD1	LEU	A	190	28.056	8.935	8.903	1.00	28.40	6	C
ATOM	1377	CD2	LEU	A	190	28.010	11.468	9.085	1.00	30.40	6	C
ATOM	1378	C	LEU	A	190	28.020	11.363	4.595	1.00	25.17	6	C
ATOM	1379	O	LEU	A	190	28.271	10.409	3.869	1.00	24.80	8	O
ATOM	1380	N	CYS	A	191	28.422	12.608	4.345	1.00	25.78	7	N
ATOM	1381	CA	CYS	A	191	29.239	12.926	3.178	1.00	26.60	6	C
ATOM	1382	CB	CYS	A	191	28.477	13.798	2.184	1.00	26.74	6	C
ATOM	1383	SG	CYS	A	191	29.522	14.300	0.771	1.00	29.77	16	S
ATOM	1384	C	CYS	A	191	30.512	13.648	3.604	1.00	26.28	6	C
ATOM	1385	O	CYS	A	191	30.464	14.735	4.154	1.00	25.81	8	O
ATOM	1386	N	PRO	A	192	31.655	13.046	3.319	1.00	26.92	7	N
ATOM	1387	CA	PRO	A	192	32.937	13.622	3.713	1.00	27.35	6	C
ATOM	1388	CB	PRO	A	192	33.899	12.459	3.531	1.00	27.11	6	C
ATOM	1389	CG	PRO	A	192	33.290	11.633	2.441	1.00	27.39	6	C
ATOM	1390	CD	PRO	A	192	31.805	11.796	2.556	1.00	26.86	6	C
ATOM	1391	C	PRO	A	192	33.352	14.774	2.789	1.00	27.90	6	C
ATOM	1392	O	PRO	A	192	34.255	15.530	3.138	1.00	28.13	8	O
ATOM	1393	N	LEU	A	193	32.717	14.895	1.630	1.00	28.55	7	N
ATOM	1394	CA	LEU	A	193	33.070	15.966	0.689	1.00	29.46	6	C
ATOM	1395	CB	LEU	A	193	32.635	15.614	-0.738	1.00	30.04	6	C
ATOM	1396	CG	LEU	A	193	33.225	14.307	-1.290	1.00	30.91	6	C
ATOM	1397	CD1	LEU	A	193	32.849	14.105	-2.754	1.00	34.39	6	C
ATOM	1398	CD2	LEU	A	193	34.738	14.306	-1.131	1.00	33.74	6	C
ATOM	1399	C	LEU	A	193	32.420	17.257	1.163	1.00	29.81	6	C
ATOM	1400	O	LEU	A	193	33.048	18.321	1.164	1.00	30.32	8	O
ATOM	1401	N	MET	A	194	31.164	17.158	1.590	1.00	29.55	7	N
ATOM	1402	CA	MET	A	194	30.470	18.308	2.158	1.00	30.51	6	C
ATOM	1403	CB	MET	A	194	28.949	18.191	1.951	1.00	30.64	6	C
ATOM	1404	CG	MET	A	194	28.497	18.114	0.492	1.00	33.89	6	C
ATOM	1405	SD	MET	A	194	28.350	19.743	-0.282	1.00	40.49	16	S
ATOM	1406	CE	MET	A	194	29.874	20.489	0.168	1.00	38.93	6	C
ATOM	1407	C	MET	A	194	30.772	18.452	3.657	1.00	29.73	6	C
ATOM	1408	O	MET	A	194	30.501	19.497	4.240	1.00	30.14	8	O
ATOM	1409	N	ALA	A	195	31.326	17.404	4.268	1.00	29.26	7	N
ATOM	1410	CA	ALA	A	195	31.543	17.367	5.722	1.00	28.29	6	C
ATOM	1411	CB	ALA	A	195	32.578	18.420	6.168	1.00	28.53	6	C
ATOM	1412	C	ALA	A	195	30.201	17.599	6.395	1.00	27.41	6	C
ATOM	1413	O	ALA	A	195	30.058	18.437	7.284	1.00	27.21	8	O
ATOM	1414	N	ALA	A	196	29.209	16.834	5.962	1.00	26.60	7	N
ATOM	1415	CA	ALA	A	196	27.846	17.048	6.403	1.00	25.81	6	C
ATOM	1416	CB	ALA	A	196	27.066	17.729	5.308	1.00	26.13	6	C

ATOM	1417	C	ALA	A	196	27.163	15.744	6.769	1.00	25.38	6	C
ATOM	1418	O	ALA	A	196	27.605	14.665	6.377	1.00	25.32	8	O
ATOM	1419	N	VAL	A	197	26.093	15.853	7.543	1.00	25.22	7	N
ATOM	1420	CA	VAL	A	197	25.289	14.691	7.888	1.00	24.87	6	C
ATOM	1421	CB	VAL	A	197	25.497	14.215	9.353	1.00	25.21	6	C
ATOM	1422	CG1	VAL	A	197	25.102	15.298	10.359	1.00	24.80	6	C
ATOM	1423	CG2	VAL	A	197	24.701	12.929	9.618	1.00	26.76	6	C
ATOM	1424	C	VAL	A	197	23.828	15.008	7.663	1.00	25.04	6	C
ATOM	1425	O	VAL	A	197	23.346	16.069	8.053	1.00	24.74	8	O
ATOM	1426	N	THR	A	198	23.120	14.077	7.027	1.00	24.63	7	N
ATOM	1427	CA	THR	A	198	21.687	14.218	6.852	1.00	25.28	6	C
ATOM	1428	CB	THR	A	198	21.309	13.955	5.385	1.00	25.27	6	C
ATOM	1429	OG1	THR	A	198	21.801	15.031	4.591	1.00	24.40	8	O
ATOM	1430	CG2	THR	A	198	19.779	14.020	5.178	1.00	25.08	6	C
ATOM	1431	C	THR	A	198	21.026	13.216	7.786	1.00	25.74	6	C
ATOM	1432	O	THR	A	198	21.331	12.032	7.743	1.00	25.91	8	O
ATOM	1433	N	TYR	A	199	20.161	13.709	8.666	1.00	26.25	7	N
ATOM	1434	CA	TYR	A	199	19.473	12.877	9.635	1.00	26.86	6	C
ATOM	1435	CB	TYR	A	199	19.636	13.490	11.034	1.00	26.82	6	C
ATOM	1436	CG	TYR	A	199	18.974	12.708	12.139	1.00	28.67	6	C
ATOM	1437	CD1	TYR	A	199	19.057	11.328	12.182	1.00	29.47	6	C
ATOM	1438	CE1	TYR	A	199	18.455	10.606	13.196	1.00	32.81	6	C
ATOM	1439	CZ	TYR	A	199	17.764	11.272	14.188	1.00	34.25	6	C
ATOM	1440	OH	TYR	A	199	17.163	10.561	15.205	1.00	38.37	8	O
ATOM	1441	CE2	TYR	A	199	17.671	12.643	14.174	1.00	33.45	6	C
ATOM	1442	CD2	TYR	A	199	18.277	13.357	13.151	1.00	30.73	6	C
ATOM	1443	C	TYR	A	199	18.000	12.780	9.268	1.00	26.79	6	C
ATOM	1444	O	TYR	A	199	17.345	13.803	9.042	1.00	26.93	8	O
ATOM	1445	N	ILE	A	200	17.502	11.550	9.168	1.00	26.74	7	N
ATOM	1446	CA	ILE	A	200	16.097	11.284	8.868	1.00	27.16	6	C
ATOM	1447	CB	ILE	A	200	15.948	10.266	7.714	1.00	26.97	6	C
ATOM	1448	CG1	ILE	A	200	16.456	10.861	6.398	1.00	26.38	6	C
ATOM	1449	CD1	ILE	A	200	16.462	9.862	5.225	1.00	26.08	6	C
ATOM	1450	CG2	ILE	A	200	14.482	9.858	7.550	1.00	26.89	6	C
ATOM	1451	C	ILE	A	200	15.545	10.696	10.157	1.00	27.75	6	C
ATOM	1452	O	ILE	A	200	15.995	9.639	10.595	1.00	27.06	8	O
ATOM	1453	N	ASP	A	201	14.605	11.393	10.790	1.00	29.03	7	N
ATOM	1454	CA	ASP	A	201	14.122	10.955	12.099	1.00	30.64	6	C
ATOM	1455	CB	ASP	A	201	13.854	12.161	13.017	1.00	31.21	6	C
ATOM	1456	CG	ASP	A	201	12.624	12.960	12.611	1.00	32.40	6	C
ATOM	1457	OD1	ASP	A	201	11.793	12.475	11.812	1.00	33.54	8	O
ATOM	1458	OD2	ASP	A	201	12.397	14.096	13.071	1.00	35.66	8	O
ATOM	1459	C	ASP	A	201	12.921	10.002	12.011	1.00	31.64	6	C
ATOM	1460	O	ASP	A	201	12.446	9.696	10.916	1.00	31.11	8	O
ATOM	1461	N	GLU	A	202	12.443	9.525	13.160	1.00	33.23	7	N
ATOM	1462	CA	GLU	A	202	11.361	8.542	13.173	1.00	34.74	6	C
ATOM	1463	CB	GLU	A	202	11.138	7.940	14.571	1.00	35.27	6	C
ATOM	1464	CG	GLU	A	202	11.226	8.923	15.722	1.00	38.23	6	C
ATOM	1465	CD	GLU	A	202	12.657	9.148	16.179	1.00	41.73	6	C
ATOM	1466	OE1	GLU	A	202	13.209	8.265	16.888	1.00	43.97	8	O
ATOM	1467	OE2	GLU	A	202	13.230	10.204	15.827	1.00	41.84	8	O
ATOM	1468	C	GLU	A	202	10.050	9.052	12.583	1.00	35.39	6	C
ATOM	1469	O	GLU	A	202	9.156	8.265	12.282	1.00	35.88	8	O
ATOM	1470	N	LYS	A	203	9.938	10.360	12.398	1.00	35.90	7	N
ATOM	1471	CA	LYS	A	203	8.740	10.919	11.790	1.00	36.58	6	C
ATOM	1472	CB	LYS	A	203	8.337	12.212	12.508	1.00	37.33	6	C
ATOM	1473	CG	LYS	A	203	8.233	12.040	14.025	1.00	38.95	6	C

ATOM	1474	CD	LYS	A	203	7.774	13.318	14.718	1.00	42.84	6	C
ATOM	1475	CE	LYS	A	203	7.529	13.084	16.207	1.00	44.43	6	C
ATOM	1476	NZ	LYS	A	203	6.740	14.186	16.831	1.00	46.60	7	N
ATOM	1477	C	LYS	A	203	8.957	11.146	10.295	1.00	36.53	6	C
ATOM	1478	O	LYS	A	203	8.072	11.626	9.594	1.00	36.51	8	O
ATOM	1479	N	ARG	A	204	10.139	10.765	9.814	1.00	36.40	7	N
ATOM	1480	CA	ARG	A	204	10.523	10.908	8.405	1.00	36.41	6	C
ATOM	1481	CB	ARG	A	204	9.467	10.330	7.468	1.00	36.80	6	C
ATOM	1482	CG	ARG	A	204	9.260	8.842	7.667	1.00	38.81	6	C
ATOM	1483	CD	ARG	A	204	8.316	8.209	6.664	1.00	42.51	6	C
ATOM	1484	NE	ARG	A	204	7.496	7.175	7.291	1.00	47.42	7	N
ATOM	1485	CZ	ARG	A	204	7.602	5.882	7.028	1.00	48.72	6	C
ATOM	1486	NH1	ARG	A	204	8.500	5.465	6.151	1.00	50.90	7	N
ATOM	1487	NH2	ARG	A	204	6.819	5.003	7.640	1.00	49.75	7	N
ATOM	1488	C	ARG	A	204	10.872	12.344	8.053	1.00	36.27	6	C
ATOM	1489	O	ARG	A	204	11.058	12.702	6.886	1.00	35.30	8	O
ATOM	1490	N	ASP	A	205	10.958	13.163	9.091	1.00	36.42	7	N
ATOM	1491	CA	ASP	A	205	11.386	14.534	8.939	1.00	37.02	6	C
ATOM	1492	CB	ASP	A	205	11.021	15.342	10.176	1.00	37.71	6	C
ATOM	1493	CG	ASP	A	205	10.499	16.710	9.831	1.00	40.93	6	C
ATOM	1494	OD1	ASP	A	205	11.285	17.681	9.909	1.00	42.87	8	O
ATOM	1495	OD2	ASP	A	205	9.316	16.901	9.460	1.00	44.32	8	O
ATOM	1496	C	ASP	A	205	12.895	14.482	8.761	1.00	36.46	6	C
ATOM	1497	O	ASP	A	205	13.564	13.562	9.247	1.00	36.31	8	O
ATOM	1498	N	PHE	A	206	13.446	15.458	8.060	1.00	35.73	7	N
ATOM	1499	CA	PHE	A	206	14.866	15.414	7.794	1.00	34.97	6	C
ATOM	1500	CB	PHE	A	206	15.096	14.828	6.403	1.00	35.12	6	C
ATOM	1501	CG	PHE	A	206	14.542	15.677	5.298	1.00	35.80	6	C
ATOM	1502	CD1	PHE	A	206	15.337	16.622	4.663	1.00	36.66	6	C
ATOM	1503	CE1	PHE	A	206	14.831	17.407	3.647	1.00	36.61	6	C
ATOM	1504	CZ	PHE	A	206	13.516	17.260	3.255	1.00	37.26	6	C
ATOM	1505	CE2	PHE	A	206	12.711	16.328	3.881	1.00	37.55	6	C
ATOM	1506	CD2	PHE	A	206	13.222	15.546	4.899	1.00	37.21	6	C
ATOM	1507	C	PHE	A	206	15.514	16.787	7.870	1.00	34.23	6	C
ATOM	1508	O	PHE	A	206	14.846	17.813	7.736	1.00	34.04	8	O
ATOM	1509	N	ARG	A	207	16.829	16.775	8.059	1.00	32.42	7	N
ATOM	1510	CA	ARG	A	207	17.639	17.973	8.080	1.00	31.15	6	C
ATOM	1511	CB	ARG	A	207	17.721	18.530	9.504	1.00	31.65	6	C
ATOM	1512	CG	ARG	A	207	17.048	19.867	9.750	1.00	34.21	6	C
ATOM	1513	CD	ARG	A	207	15.763	20.088	9.013	1.00	37.48	6	C
ATOM	1514	NE	ARG	A	207	14.911	21.073	9.676	1.00	39.43	7	N
ATOM	1515	CZ	ARG	A	207	13.596	20.943	9.761	1.00	40.61	6	C
ATOM	1516	NH1	ARG	A	207	13.009	19.882	9.221	1.00	40.43	7	N
ATOM	1517	NH2	ARG	A	207	12.865	21.865	10.375	1.00	41.27	7	N
ATOM	1518	C	ARG	A	207	19.044	17.589	7.646	1.00	29.46	6	C
ATOM	1519	O	ARG	A	207	19.516	16.488	7.939	1.00	28.39	8	O
ATOM	1520	N	THR	A	208	19.717	18.509	6.973	1.00	27.70	7	N
ATOM	1521	CA	THR	A	208	21.117	18.324	6.635	1.00	26.90	6	C
ATOM	1522	CB	THR	A	208	21.340	18.595	5.155	1.00	27.18	6	C
ATOM	1523	OG1	THR	A	208	20.704	17.563	4.396	1.00	28.05	8	O
ATOM	1524	CG2	THR	A	208	22.824	18.467	4.810	1.00	26.41	6	C
ATOM	1525	C	THR	A	208	21.914	19.317	7.477	1.00	26.37	6	C
ATOM	1526	O	THR	A	208	21.589	20.491	7.498	1.00	26.33	8	O
ATOM	1527	N	TYR	A	209	22.938	18.837	8.179	1.00	25.78	7	N
ATOM	1528	CA	TYR	A	209	23.731	19.689	9.074	1.00	25.54	6	C
ATOM	1529	CB	TYR	A	209	23.648	19.141	10.505	1.00	25.31	6	C
ATOM	1530	CG	TYR	A	209	22.274	19.111	11.095	1.00	25.56	6	C

```
ATOM   1531  CD1 TYR A 209      21.540  17.936  11.127  1.00 27.19      6      C
ATOM   1532  CE1 TYR A 209      20.278  17.897  11.678  1.00 27.25      6      C
ATOM   1533  CZ  TYR A 209      19.731  19.048  12.198  1.00 27.58      6      C
ATOM   1534  OH  TYR A 209      18.471  19.004  12.741  1.00 28.08      8      O
ATOM   1535  CE2 TYR A 209      20.439  20.235  12.180  1.00 27.33      6      C
ATOM   1536  CD2 TYR A 209      21.705  20.259  11.632  1.00 27.03      6      C
ATOM   1537  C   TYR A 209      25.199  19.673   8.713  1.00 25.06      6      C
ATOM   1538  O   TYR A 209      25.746  18.617   8.387  1.00 24.78      8      O
ATOM   1539  N   ARG A 210      25.856  20.828   8.799  1.00 24.92      7      N
ATOM   1540  CA  ARG A 210      27.298  20.864   8.640  1.00 24.96      6      C
ATOM   1541  CB  ARG A 210      27.773  22.300   8.432  1.00 25.52      6      C
ATOM   1542  CG  ARG A 210      28.709  22.478   7.288  1.00 29.28      6      C
ATOM   1543  CD  ARG A 210      28.950  23.949   6.924  1.00 31.30      6      C
ATOM   1544  NE  ARG A 210      28.560  24.209   5.547  1.00 36.97      7      N
ATOM   1545  CZ  ARG A 210      29.356  24.017   4.512  1.00 37.48      6      C
ATOM   1546  NH1 ARG A 210      30.595  23.578   4.709  1.00 38.89      7      N
ATOM   1547  NH2 ARG A 210      28.925  24.276   3.294  1.00 37.04      7      N
ATOM   1548  C   ARG A 210      27.877  20.350   9.951  1.00 24.74      6      C
ATOM   1549  O   ARG A 210      27.537  20.862  11.030  1.00 24.12      8      O
ATOM   1550  N   LEU A 211      28.762  19.362   9.867  1.00 23.95      7      N
ATOM   1551  CA  LEU A 211      29.324  18.756  11.076  1.00 24.48      6      C
ATOM   1552  CB  LEU A 211      30.228  17.573  10.714  1.00 24.81      6      C
ATOM   1553  CG  LEU A 211      29.472  16.344  10.216  1.00 25.82      6      C
ATOM   1554  CD1 LEU A 211      30.420  15.356   9.535  1.00 28.04      6      C
ATOM   1555  CD2 LEU A 211      28.746  15.673  11.405  1.00 27.62      6      C
ATOM   1556  C   LEU A 211      30.085  19.742  11.950  1.00 24.79      6      C
ATOM   1557  O   LEU A 211      29.979  19.701  13.183  1.00 24.62      8      O
ATOM   1558  N   SER A 212      30.859  20.623  11.323  1.00 24.24      7      N
ATOM   1559  CA  SER A 212      31.621  21.621  12.080  1.00 24.90      6      C
ATOM   1560  CB  SER A 212      32.659  22.353  11.195  1.00 24.80      6      C
ATOM   1561  OG  SER A 212      32.048  23.064  10.141  1.00 25.93      8      O
ATOM   1562  C   SER A 212      30.704  22.602  12.816  1.00 24.67      6      C
ATOM   1563  O   SER A 212      31.068  23.094  13.880  1.00 24.77      8      O
ATOM   1564  N   LEU A 213      29.507  22.855  12.286  1.00 24.57      7      N
ATOM   1565  CA  LEU A 213      28.556  23.740  12.974  1.00 24.86      6      C
ATOM   1566  CB  LEU A 213      27.526  24.316  12.002  1.00 24.39      6      C
ATOM   1567  CG  LEU A 213      28.111  25.341  11.026  1.00 24.18      6      C
ATOM   1568  CD1 LEU A 213      27.047  25.832  10.040  1.00 23.86      6      C
ATOM   1569  CD2 LEU A 213      28.745  26.527  11.790  1.00 24.15      6      C
ATOM   1570  C   LEU A 213      27.855  23.053  14.151  1.00 25.20      6      C
ATOM   1571  O   LEU A 213      27.463  23.714  15.123  1.00 24.93      8      O
ATOM   1572  N   LEU A 214      27.672  21.737  14.059  1.00 25.82      7      N
ATOM   1573  CA  LEU A 214      27.120  20.975  15.197  1.00 26.69      6      C
ATOM   1574  CB  LEU A 214      26.877  19.513  14.812  1.00 26.57      6      C
ATOM   1575  CG  LEU A 214      25.718  19.265  13.847  1.00 26.82      6      C
ATOM   1576  CD1 LEU A 214      25.672  17.794  13.400  1.00 26.32      6      C
ATOM   1577  CD2 LEU A 214      24.401  19.674  14.490  1.00 26.19      6      C
ATOM   1578  C   LEU A 214      28.132  21.043  16.335  1.00 27.38      6      C
ATOM   1579  O   LEU A 214      27.778  21.077  17.525  1.00 27.59      8      O
ATOM   1580  N   GLU A 215      29.405  21.044  15.961  1.00 27.94      7      N
ATOM   1581  CA  GLU A 215      30.480  21.129  16.938  1.00 29.14      6      C
ATOM   1582  CB  GLU A 215      31.832  20.999  16.233  1.00 29.52      6      C
ATOM   1583  CG  GLU A 215      33.024  21.144  17.151  1.00 31.92      6      C
ATOM   1584  CD  GLU A 215      34.330  20.820  16.457  1.00 35.42      6      C
ATOM   1585  OE1 GLU A 215      34.317  20.077  15.442  1.00 37.83      8      O
ATOM   1586  OE2 GLU A 215      35.371  21.311  16.932  1.00 38.01      8      O
ATOM   1587  C   GLU A 215      30.398  22.459  17.681  1.00 29.04      6      C
```

ATOM	1588	O	GLU	A	215	30.588	22.537	18.901	1.00	28.72	8	O
ATOM	1589	N	GLU	A	216	30.074	23.512	16.939	1.00	28.90	7	N
ATOM	1590	CA	GLU	A	216	30.031	24.846	17.518	1.00	28.96	6	C
ATOM	1591	CB	GLU	A	216	30.291	25.894	16.435	1.00	29.27	6	C
ATOM	1592	CG	GLU	A	216	31.704	25.831	15.898	1.00	32.49	6	C
ATOM	1593	CD	GLU	A	216	31.881	26.639	14.634	1.00	35.60	6	C
ATOM	1594	OE1	GLU	A	216	31.369	27.788	14.599	1.00	31.06	8	O
ATOM	1595	OE2	GLU	A	216	32.525	26.100	13.693	1.00	36.77	8	O
ATOM	1596	C	GLU	A	216	28.728	25.158	18.229	1.00	28.41	6	C
ATOM	1597	O	GLU	A	216	28.737	25.791	19.282	1.00	27.84	8	O
ATOM	1598	N	TYR	A	217	27.611	24.686	17.675	1.00	28.11	7	N
ATOM	1599	CA	TYR	A	217	26.295	25.039	18.201	1.00	28.19	6	C
ATOM	1600	CB	TYR	A	217	25.402	25.548	17.070	1.00	28.42	6	C
ATOM	1601	CG	TYR	A	217	25.858	26.892	16.546	1.00	29.01	6	C
ATOM	1602	CD1	TYR	A	217	26.601	26.996	15.375	1.00	30.89	6	C
ATOM	1603	CE1	TYR	A	217	27.031	28.241	14.908	1.00	31.06	6	C
ATOM	1604	CZ	TYR	A	217	26.711	29.381	15.631	1.00	31.94	6	C
ATOM	1605	OH	TYR	A	217	27.125	30.617	15.199	1.00	32.46	8	O
ATOM	1606	CE2	TYR	A	217	25.980	29.288	16.794	1.00	30.74	6	C
ATOM	1607	CD2	TYR	A	217	25.557	28.057	17.240	1.00	30.04	6	C
ATOM	1608	C	TYR	A	217	25.578	23.951	19.007	1.00	28.54	6	C
ATOM	1609	O	TYR	A	217	24.570	24.239	19.662	1.00	28.35	8	O
ATOM	1610	N	GLY	A	218	26.092	22.723	18.947	1.00	28.00	7	N
ATOM	1611	CA	GLY	A	218	25.524	21.602	19.696	1.00	28.61	6	C
ATOM	1612	C	GLY	A	218	24.399	20.886	18.972	1.00	28.81	6	C
ATOM	1613	O	GLY	A	218	23.959	21.325	17.914	1.00	28.46	8	O
ATOM	1614	N	CYS	A	219	23.937	19.770	19.535	1.00	29.39	7	N
ATOM	1615	CA	CYS	A	219	22.804	19.047	18.974	1.00	30.37	6	C
ATOM	1616	CB	CYS	A	219	23.192	18.175	17.771	1.00	30.56	6	C
ATOM	1617	SG	CYS	A	219	24.248	16.767	18.144	1.00	32.81	16	S
ATOM	1618	C	CYS	A	219	22.139	18.210	20.045	1.00	30.63	6	C
ATOM	1619	O	CYS	A	219	22.665	18.061	21.146	1.00	30.45	8	O
ATOM	1620	N	CYS	A	220	20.978	17.661	19.719	1.00	31.60	7	N
ATOM	1621	CA	CYS	A	220	20.224	16.889	20.695	1.00	32.42	6	C
ATOM	1622	CB	CYS	A	220	18.800	16.657	20.189	1.00	32.94	6	C
ATOM	1623	SG	CYS	A	220	18.712	15.547	18.750	1.00	35.67	16	S
ATOM	1624	C	CYS	A	220	20.877	15.543	20.957	1.00	32.66	6	C
ATOM	1625	O	CYS	A	220	21.647	15.042	20.142	1.00	31.36	8	O
ATOM	1626	N	LYS	A	221	20.593	14.975	22.123	1.00	33.15	7	N
ATOM	1627	CA	LYS	A	221	20.997	13.620	22.378	1.00	34.52	6	C
ATOM	1628	CB	LYS	A	221	20.503	13.168	23.762	1.00	35.08	6	C
ATOM	1629	CG	LYS	A	221	20.396	11.664	23.931	1.00	37.45	6	C
ATOM	1630	CD	LYS	A	221	19.537	11.281	25.137	1.00	41.50	6	C
ATOM	1631	CE	LYS	A	221	19.220	9.793	25.114	1.00	43.16	6	C
ATOM	1632	NZ	LYS	A	221	18.022	9.437	25.937	1.00	44.32	7	N
ATOM	1633	C	LYS	A	221	20.200	12.966	21.261	1.00	34.63	6	C
ATOM	1634	O	LYS	A	221	19.268	13.553	20.750	1.00	36.08	8	O
ATOM	1635	N	GLU	A	222	20.543	11.777	20.837	1.00	34.66	7	N
ATOM	1636	CA	GLU	A	222	19.779	11.175	19.742	1.00	33.55	6	C
ATOM	1637	CB	GLU	A	222	18.390	11.802	19.530	1.00	34.71	6	C
ATOM	1638	CG	GLU	A	222	17.261	11.284	20.433	1.00	37.70	6	C
ATOM	1639	CD	GLU	A	222	16.841	12.297	21.487	1.00	41.31	6	C
ATOM	1640	OE1	GLU	A	222	17.183	13.496	21.338	1.00	40.54	8	O
ATOM	1641	OE2	GLU	A	222	16.163	11.901	22.471	1.00	44.12	8	O
ATOM	1642	C	GLU	A	222	20.601	11.365	18.494	1.00	31.77	6	C
ATOM	1643	O	GLU	A	222	21.102	10.399	17.961	1.00	31.65	8	O
ATOM	1644	N	LEU	A	223	20.737	12.600	18.011	1.00	30.02	7	N

ATOM	1645	CA	LEU	A	223	21.627	12.797	16.869	1.00	28.45	6	C
ATOM	1646	CB	LEU	A	223	21.482	14.186	16.236	1.00	28.53	6	C
ATOM	1647	CG	LEU	A	223	22.461	14.498	15.100	1.00	28.64	6	C
ATOM	1648	CD1	LEU	A	223	22.416	13.409	14.023	1.00	28.96	6	C
ATOM	1649	CD2	LEU	A	223	22.160	15.859	14.506	1.00	28.18	6	C
ATOM	1650	C	LEU	A	223	23.044	12.546	17.374	1.00	27.23	6	C
ATOM	1651	O	LEU	A	223	23.826	11.848	16.738	1.00	25.93	8	O
ATOM	1652	N	ALA	A	224	23.356	13.073	18.559	1.00	26.68	7	N
ATOM	1653	CA	ALA	A	224	24.683	12.874	19.127	1.00	26.35	6	C
ATOM	1654	CB	ALA	A	224	24.822	13.605	20.474	1.00	26.82	6	C
ATOM	1655	C	ALA	A	224	25.033	11.405	19.292	1.00	25.93	6	C
ATOM	1656	O	ALA	A	224	26.161	10.988	19.001	1.00	24.68	8	O
ATOM	1657	N	SER	A	225	24.076	10.618	19.772	1.00	25.86	7	N
ATOM	1658	CA	SER	A	225	24.341	9.209	20.004	1.00	26.40	6	C
ATOM	1659	CB	SER	A	225	23.220	8.566	20.831	1.00	26.51	6	C
ATOM	1660	OG	SER	A	225	21.999	8.630	20.130	1.00	31.54	8	O
ATOM	1661	C	SER	A	225	24.520	8.478	18.670	1.00	25.53	6	C
ATOM	1662	O	SER	A	225	25.331	7.550	18.559	1.00	25.79	8	O
ATOM	1663	N	ARG	A	226	23.759	8.893	17.668	1.00	24.77	7	N
ATOM	1664	CA	ARG	A	226	23.867	8.272	16.354	1.00	24.66	6	C
ATOM	1665	CB	ARG	A	226	22.660	8.614	15.485	1.00	24.86	6	C
ATOM	1666	CG	ARG	A	226	21.403	7.808	15.900	1.00	25.80	6	C
ATOM	1667	CD	ARG	A	226	20.076	8.383	15.422	1.00	27.28	6	C
ATOM	1668	NE	ARG	A	226	18.934	7.584	15.889	1.00	27.33	7	N
ATOM	1669	CZ	ARG	A	226	18.443	7.622	17.129	1.00	29.35	6	C
ATOM	1670	NH1	ARG	A	226	18.974	8.429	18.041	1.00	28.48	7	N
ATOM	1671	NH2	ARG	A	226	17.403	6.864	17.458	1.00	30.48	7	N
ATOM	1672	C	ARG	A	226	25.202	8.615	15.686	1.00	24.36	6	C
ATOM	1673	O	ARG	A	226	25.783	7.777	14.985	1.00	24.15	8	O
ATOM	1674	N	LEU	A	227	25.687	9.835	15.915	1.00	24.00	7	N
ATOM	1675	CA	LEU	A	227	26.993	10.243	15.385	1.00	24.49	6	C
ATOM	1676	CB	LEU	A	227	27.197	11.751	15.522	1.00	25.07	6	C
ATOM	1677	CG	LEU	A	227	26.409	12.631	14.546	1.00	25.52	6	C
ATOM	1678	CD1	LEU	A	227	26.575	14.116	14.901	1.00	25.92	6	C
ATOM	1679	CD2	LEU	A	227	26.832	12.394	13.087	1.00	27.53	6	C
ATOM	1680	C	LEU	A	227	28.139	9.470	16.066	1.00	24.12	6	C
ATOM	1681	O	LEU	A	227	29.154	9.164	15.436	1.00	23.19	8	O
ATOM	1682	N	ARG	A	228	27.981	9.151	17.351	1.00	23.81	7	N
ATOM	1683	CA	ARG	A	228	28.973	8.310	18.026	1.00	24.03	6	C
ATOM	1684	CB	ARG	A	228	28.654	8.178	19.526	1.00	23.73	6	C
ATOM	1685	CG	ARG	A	228	29.133	9.349	20.409	1.00	26.03	6	C
ATOM	1686	CD	ARG	A	228	28.765	9.173	21.909	1.00	31.16	6	C
ATOM	1687	NE	ARG	A	228	27.857	10.250	22.283	1.00	36.96	7	N
ATOM	1688	CZ	ARG	A	228	26.632	10.098	22.737	1.00	36.30	6	C
ATOM	1689	NH1	ARG	A	228	26.118	8.889	22.949	1.00	37.17	7	N
ATOM	1690	NH2	ARG	A	228	25.928	11.174	23.012	1.00	38.46	7	N
ATOM	1691	C	ARG	A	228	28.992	6.929	17.375	1.00	23.69	6	C
ATOM	1692	O	ARG	A	228	30.053	6.366	17.098	1.00	23.53	8	O
ATOM	1693	N	TYR	A	229	27.805	6.371	17.157	1.00	24.48	7	N
ATOM	1694	CA	TYR	A	229	27.673	5.069	16.509	1.00	24.19	6	C
ATOM	1695	CB	TYR	A	229	26.196	4.703	16.373	1.00	24.66	6	C
ATOM	1696	CG	TYR	A	229	25.971	3.265	15.968	1.00	25.51	6	C
ATOM	1697	CD1	TYR	A	229	25.931	2.264	16.930	1.00	26.27	6	C
ATOM	1698	CE1	TYR	A	229	25.730	0.944	16.580	1.00	28.27	6	C
ATOM	1699	CZ	TYR	A	229	25.563	0.598	15.258	1.00	26.61	6	C
ATOM	1700	OH	TYR	A	229	25.367	-0.732	14.947	1.00	27.01	8	O
ATOM	1701	CE2	TYR	A	229	25.601	1.561	14.274	1.00	27.00	6	C

```
ATOM   1702  CD2 TYR A 229      25.807   2.902  14.628  1.00 25.01    6        C
ATOM   1703  C   TYR A 229      28.312   5.117  15.110  1.00 23.94    6        C
ATOM   1704  O   TYR A 229      29.032   4.211  14.713  1.00 23.19    8        O
ATOM   1705  N   ALA A 230      28.044   6.190  14.376  1.00 23.63    7        N
ATOM   1706  CA  ALA A 230      28.627   6.350  13.033  1.00 23.80    6        C
ATOM   1707  CB  ALA A 230      28.128   7.634  12.391  1.00 23.61    6        C
ATOM   1708  C   ALA A 230      30.165   6.297  13.042  1.00 23.70    6        C
ATOM   1709  O   ALA A 230      30.790   5.651  12.186  1.00 23.89    8        O
ATOM   1710  N   ARG A 231      30.780   6.956  14.016  1.00 23.74    7        N
ATOM   1711  CA  ARG A 231      32.234   6.917  14.138  1.00 24.06    6        C
ATOM   1712  CB  ARG A 231      32.710   7.811  15.283  1.00 24.34    6        C
ATOM   1713  CG  ARG A 231      34.223   7.959  15.371  1.00 26.14    6        C
ATOM   1714  CD  ARG A 231      34.902   7.010  16.354  1.00 28.93    6        C
ATOM   1715  NE  ARG A 231      36.355   7.205  16.370  1.00 31.60    7        N
ATOM   1716  CZ  ARG A 231      36.961   8.234  16.942  1.00 33.08    6        C
ATOM   1717  NH1 ARG A 231      36.251   9.163  17.571  1.00 34.81    7        N
ATOM   1718  NH2 ARG A 231      38.283   8.339  16.895  1.00 35.10    7        N
ATOM   1719  C   ARG A 231      32.755   5.487  14.313  1.00 23.52    6        C
ATOM   1720  O   ARG A 231      33.743   5.103  13.686  1.00 24.27    8        O
ATOM   1721  N   THR A 232      32.084   4.707  15.155  1.00 23.50    7        N
ATOM   1722  CA  THR A 232      32.442   3.312  15.370  1.00 23.31    6        C
ATOM   1723  CB  THR A 232      31.491   2.700  16.424  1.00 24.05    6        C
ATOM   1724  OG1 THR A 232      31.636   3.415  17.666  1.00 24.01    8        O
ATOM   1725  CG2 THR A 232      31.905   1.260  16.755  1.00 24.30    6        C
ATOM   1726  C   THR A 232      32.330   2.533  14.056  1.00 23.53    6        C
ATOM   1727  O   THR A 232      33.188   1.705  13.718  1.00 22.83    8        O
ATOM   1728  N   MET A 233      31.258   2.792  13.317  1.00 23.02    7        N
ATOM   1729  CA  MET A 233      31.071   2.114  12.042  1.00 23.97    6        C
ATOM   1730  CB  MET A 233      29.681   2.389  11.474  1.00 23.70    6        C
ATOM   1731  CG  MET A 233      28.548   1.893  12.339  1.00 24.89    6        C
ATOM   1732  SD  MET A 233      28.603   0.124  12.651  1.00 25.92   16        S
ATOM   1733  CE  MET A 233      29.192   0.099  14.342  1.00 27.03    6        C
ATOM   1734  C   MET A 233      32.141   2.509  11.032  1.00 23.90    6        C
ATOM   1735  O   MET A 233      32.602   1.671  10.255  1.00 24.29    8        O
ATOM   1736  N   VAL A 234      32.528   3.779  11.034  1.00 24.08    7        N
ATOM   1737  CA  VAL A 234      33.561   4.232  10.099  1.00 25.69    6        C
ATOM   1738  CB  VAL A 234      33.647   5.767  10.036  1.00 25.75    6        C
ATOM   1739  CG1 VAL A 234      34.896   6.230   9.251  1.00 26.58    6        C
ATOM   1740  CG2 VAL A 234      32.379   6.325   9.415  1.00 26.02    6        C
ATOM   1741  C   VAL A 234      34.912   3.586  10.434  1.00 26.22    6        C
ATOM   1742  O   VAL A 234      35.628   3.137   9.531  1.00 27.03    8        O
ATOM   1743  N   ASP A 235      35.242   3.502  11.723  1.00 27.15    7        N
ATOM   1744  CA  ASP A 235      36.470   2.813  12.153  1.00 27.94    6        C
ATOM   1745  CB  ASP A 235      36.618   2.810  13.685  1.00 28.58    6        C
ATOM   1746  CG  ASP A 235      37.272   4.061  14.226  1.00 30.71    6        C
ATOM   1747  OD1 ASP A 235      38.060   4.718  13.498  1.00 31.20    8        O
ATOM   1748  OD2 ASP A 235      37.073   4.460  15.397  1.00 33.27    8        O
ATOM   1749  C   ASP A 235      36.458   1.372  11.655  1.00 27.90    6        C
ATOM   1750  O   ASP A 235      37.482   0.848  11.198  1.00 27.86    8        O
ATOM   1751  N   LYS A 236      35.301   0.718  11.758  1.00 28.17    7        N
ATOM   1752  CA  LYS A 236      35.167  -0.661  11.292  1.00 29.21    6        C
ATOM   1753  CB  LYS A 236      33.805  -1.255  11.651  1.00 28.80    6        C
ATOM   1754  CG  LYS A 236      33.766  -2.755  11.453  1.00 31.44    6        C
ATOM   1755  CD  LYS A 236      32.463  -3.365  11.880  1.00 33.65    6        C
ATOM   1756  CE  LYS A 236      32.677  -4.762  12.424  1.00 35.73    6        C
ATOM   1757  NZ  LYS A 236      33.676  -5.582  11.682  1.00 33.42    7        N
ATOM   1758  C   LYS A 236      35.412  -0.779   9.781  1.00 29.51    6        C
```

ATOM	1759	O	LYS	A	236	36.136	-1.676	9.336	1.00	29.82	8	O
ATOM	1760	N	LEU	A	237	34.813	0.123	9.006	1.00	29.66	7	N
ATOM	1761	CA	LEU	A	237	35.000	0.144	7.556	1.00	30.62	6	C
ATOM	1762	CB	LEU	A	237	34.222	1.310	6.923	1.00	29.80	6	C
ATOM	1763	CG	LEU	A	237	32.701	1.153	6.793	1.00	28.82	6	C
ATOM	1764	CD1	LEU	A	237	32.026	2.447	6.386	1.00	29.45	6	C
ATOM	1765	CD2	LEU	A	237	32.362	0.031	5.797	1.00	28.42	6	C
ATOM	1766	C	LEU	A	237	36.488	0.282	7.234	1.00	31.81	6	C
ATOM	1767	O	LEU	A	237	37.002	-0.373	6.326	1.00	31.85	8	O
ATOM	1768	N	LEU	A	238	37.174	1.133	7.986	1.00	33.83	7	N
ATOM	1769	CA	LEU	A	238	38.610	1.323	7.794	1.00	36.16	6	C
ATOM	1770	CB	LEU	A	238	39.098	2.564	8.539	1.00	35.64	6	C
ATOM	1771	CG	LEU	A	238	38.733	3.870	7.834	1.00	35.77	6	C
ATOM	1772	CD1	LEU	A	238	38.772	5.047	8.792	1.00	35.86	6	C
ATOM	1773	CD2	LEU	A	238	39.644	4.104	6.622	1.00	35.95	6	C
ATOM	1774	C	LEU	A	238	39.427	0.110	8.219	1.00	37.98	6	C
ATOM	1775	O	LEU	A	238	40.483	-0.154	7.650	1.00	38.49	8	O
ATOM	1776	N	SER	A	239	38.939	-0.627	9.210	1.00	40.20	7	N
ATOM	1777	CA	SER	A	239	39.658	-1.793	9.715	1.00	42.61	6	C
ATOM	1778	CB	SER	A	239	39.069	-2.256	11.048	1.00	42.53	6	C
ATOM	1779	OG	SER	A	239	37.938	-3.081	10.828	1.00	41.85	8	O
ATOM	1780	C	SER	A	239	39.597	-2.942	8.723	1.00	44.55	6	C
ATOM	1781	O	SER	A	239	40.564	-3.680	8.551	1.00	45.33	8	O
ATOM	1782	N	SER	A	240	38.448	-3.099	8.076	1.00	46.75	7	N
ATOM	1783	CA	SER	A	240	38.266	-4.176	7.118	1.00	48.84	6	C
ATOM	1784	CB	SER	A	240	36.812	-4.656	7.104	1.00	48.94	6	C
ATOM	1785	OG	SER	A	240	35.913	-3.574	6.949	1.00	50.43	8	O
ATOM	1786	C	SER	A	240	38.705	-3.724	5.734	1.00	49.93	6	C
ATOM	1787	O	SER	A	240	38.692	-4.508	4.790	1.00	50.59	8	O
ATOM	1788	N	ALA	A	241	39.105	-2.458	5.635	1.00	51.23	7	N
ATOM	1789	CA	ALA	A	241	39.580	-1.867	4.382	1.00	52.19	6	C
ATOM	1790	CB	ALA	A	241	40.848	-1.074	4.625	1.00	52.24	6	C
ATOM	1791	C	ALA	A	241	39.819	-2.906	3.294	1.00	52.79	6	C
ATOM	1792	O	ALA	A	241	40.907	-3.488	3.238	1.00	53.19	8	O
ATOM	1793	OXT	ALA	A	241	38.934	-3.162	2.470	1.00	53.32	8	O
ATOM	1794	N	ALA	B	20	-18.462	10.374	-32.692	1.00	40.15	7	N
ATOM	1795	CA	ALA	B	20	-18.787	10.792	-31.295	1.00	39.44	6	C
ATOM	1796	CB	ALA	B	20	-19.597	12.064	-31.300	1.00	39.55	6	C
ATOM	1797	C	ALA	B	20	-19.538	9.676	-30.576	1.00	38.88	6	C
ATOM	1798	O	ALA	B	20	-20.122	8.802	-31.212	1.00	38.93	8	O
ATOM	1799	N	LEU	B	21	-19.515	9.710	-29.249	1.00	38.33	7	N
ATOM	1800	CA	LEU	B	21	-20.162	8.679	-28.452	1.00	37.95	6	C
ATOM	1801	CB	LEU	B	21	-19.852	8.865	-26.969	1.00	38.46	6	C
ATOM	1802	CG	LEU	B	21	-18.459	8.484	-26.486	1.00	39.45	6	C
ATOM	1803	CD1	LEU	B	21	-18.389	8.609	-24.970	1.00	40.66	6	C
ATOM	1804	CD2	LEU	B	21	-18.114	7.069	-26.923	1.00	40.86	6	C
ATOM	1805	C	LEU	B	21	-21.663	8.693	-28.674	1.00	37.29	6	C
ATOM	1806	O	LEU	B	21	-22.288	7.643	-28.802	1.00	36.75	8	O
ATOM	1807	N	SER	B	22	-22.235	9.894	-28.706	1.00	36.31	7	N
ATOM	1808	CA	SER	B	22	-23.662	10.057	-28.948	1.00	35.97	6	C
ATOM	1809	CB	SER	B	22	-24.027	11.551	-28.960	1.00	36.01	6	C
ATOM	1810	OG	SER	B	22	-25.386	11.740	-29.301	1.00	38.45	8	O
ATOM	1811	C	SER	B	22	-24.081	9.355	-30.248	1.00	34.55	6	C
ATOM	1812	O	SER	B	22	-25.046	8.597	-30.264	1.00	34.41	8	O
ATOM	1813	N	ASP	B	23	-23.346	9.583	-31.332	1.00	33.54	7	N
ATOM	1814	CA	ASP	B	23	-23.635	8.906	-32.596	1.00	32.88	6	C
ATOM	1815	CB	ASP	B	23	-22.679	9.371	-33.696	1.00	32.93	6	C

```
ATOM   1816  CG  ASP B  23     -22.915  10.820 -34.120  1.00 34.64   6       C
ATOM   1817  OD1 ASP B  23     -24.013  11.362 -33.878  1.00 34.23   8       O
ATOM   1818  OD2 ASP B  23     -22.050  11.485 -34.718  1.00 35.74   8       O
ATOM   1819  C   ASP B  23     -23.534   7.375 -32.453  1.00 32.47   6       C
ATOM   1820  O   ASP B  23     -24.386   6.633 -32.945  1.00 31.81   8       O
ATOM   1821  N   MET B  24     -22.490   6.906 -31.781  1.00 31.81   7       N
ATOM   1822  CA  MET B  24     -22.307   5.459 -31.625  1.00 32.01   6       C
ATOM   1823  CB  MET B  24     -20.997   5.143 -30.894  1.00 32.02   6       C
ATOM   1824  CG  MET B  24     -20.641   3.656 -30.899  1.00 33.91   6       C
ATOM   1825  SD  MET B  24     -19.040   3.300 -30.170  1.00 36.01   16      S
ATOM   1826  CE  MET B  24     -17.947   3.957 -31.436  1.00 36.64   6       C
ATOM   1827  C   MET B  24     -23.492   4.849 -30.882  1.00 31.30   6       C
ATOM   1828  O   MET B  24     -23.984   3.784 -31.245  1.00 31.16   8       O
ATOM   1829  N   LEU B  25     -23.956   5.539 -29.846  1.00 31.37   7       N
ATOM   1830  CA  LEU B  25     -25.086   5.057 -29.060  1.00 31.48   6       C
ATOM   1831  CB  LEU B  25     -25.356   5.979 -27.871  1.00 31.48   6       C
ATOM   1832  CG  LEU B  25     -26.522   5.539 -26.982  1.00 31.97   6       C
ATOM   1833  CD1 LEU B  25     -26.211   4.186 -26.338  1.00 32.19   6       C
ATOM   1834  CD2 LEU B  25     -26.852   6.594 -25.919  1.00 34.03   6       C
ATOM   1835  C   LEU B  25     -26.333   4.927 -29.928  1.00 31.62   6       C
ATOM   1836  O   LEU B  25     -27.015   3.904 -29.895  1.00 31.80   8       O
ATOM   1837  N   GLN B  26     -26.619   5.949 -30.726  1.00 31.03   7       N
ATOM   1838  CA  GLN B  26     -27.785   5.895 -31.608  1.00 30.78   6       C
ATOM   1839  CB  GLN B  26     -27.993   7.236 -32.330  1.00 31.06   6       C
ATOM   1840  CG  GLN B  26     -28.570   8.351 -31.445  1.00 33.92   6       C
ATOM   1841  CD  GLN B  26     -28.926   9.607 -32.236  1.00 38.19   6       C
ATOM   1842  OE1 GLN B  26     -28.178  10.021 -33.114  1.00 39.61   8       O
ATOM   1843  NE2 GLN B  26     -30.068  10.214 -31.919  1.00 40.60   7       N
ATOM   1844  C   GLN B  26     -27.678   4.750 -32.620  1.00 29.63   6       C
ATOM   1845  O   GLN B  26     -28.666   4.101 -32.938  1.00 29.49   8       O
ATOM   1846  N   GLN B  27     -26.482   4.524 -33.146  1.00 29.11   7       N
ATOM   1847  CA  GLN B  27     -26.258   3.449 -34.102  1.00 28.58   6       C
ATOM   1848  CB  GLN B  27     -24.844   3.560 -34.694  1.00 28.95   6       C
ATOM   1849  CG  GLN B  27     -24.627   4.822 -35.541  1.00 30.01   6       C
ATOM   1850  CD  GLN B  27     -23.158   5.178 -35.722  1.00 31.97   6       C
ATOM   1851  OE1 GLN B  27     -22.277   4.377 -35.412  1.00 30.66   8       O
ATOM   1852  NE2 GLN B  27     -22.894   6.381 -36.235  1.00 30.20   7       N
ATOM   1853  C   GLN B  27     -26.462   2.070 -33.454  1.00 28.10   6       C
ATOM   1854  O   GLN B  27     -27.047   1.168 -34.050  1.00 27.55   8       O
ATOM   1855  N   LEU B  28     -25.953   1.907 -32.239  1.00 27.60   7       N
ATOM   1856  CA  LEU B  28     -26.105   0.640 -31.534  1.00 27.94   6       C
ATOM   1857  CB  LEU B  28     -25.145   0.574 -30.344  1.00 27.52   6       C
ATOM   1858  CG  LEU B  28     -23.674   0.414 -30.741  1.00 27.57   6       C
ATOM   1859  CD1 LEU B  28     -22.758   0.627 -29.547  1.00 29.37   6       C
ATOM   1860  CD2 LEU B  28     -23.410  -0.943 -31.367  1.00 28.78   6       C
ATOM   1861  C   LEU B  28     -27.560   0.445 -31.114  1.00 28.02   6       C
ATOM   1862  O   LEU B  28     -28.134  -0.632 -31.298  1.00 28.14   8       O
ATOM   1863  N   HIS B  29     -28.172   1.498 -30.580  1.00 28.71   7       N
ATOM   1864  CA  HIS B  29     -29.567   1.411 -30.178  1.00 29.41   6       C
ATOM   1865  CB  HIS B  29     -30.088   2.755 -29.660  1.00 29.83   6       C
ATOM   1866  CG  HIS B  29     -31.563   2.756 -29.388  1.00 31.05   6       C
ATOM   1867  ND1 HIS B  29     -32.112   2.192 -28.256  1.00 33.08   7       N
ATOM   1868  CE1 HIS B  29     -33.427   2.325 -28.291  1.00 33.88   6       C
ATOM   1869  NE2 HIS B  29     -33.751   2.954 -29.408  1.00 33.28   7       N
ATOM   1870  CD2 HIS B  29     -32.604   3.236 -30.111  1.00 33.23   6       C
ATOM   1871  C   HIS B  29     -30.406   0.949 -31.363  1.00 29.61   6       C
ATOM   1872  O   HIS B  29     -31.248   0.059 -31.243  1.00 29.22   8       O
```

ATOM	1873	N	SER	B	30	-30.169	1.556	-32.516	1.00	29.41	7	N
ATOM	1874	CA	SER	B	30	-30.928	1.205	-33.710	1.00	30.33	6	C
ATOM	1875	CB	SER	B	30	-30.558	2.150	-34.857	1.00	30.29	6	C
ATOM	1876	OG	SER	B	30	-31.372	1.911	-35.981	1.00	32.27	8	O
ATOM	1877	C	SER	B	30	-30.747	-0.256	-34.145	1.00	29.67	6	C
ATOM	1878	O	SER	B	30	-31.725	-0.971	-34.379	1.00	29.62	8	O
ATOM	1879	N	VAL	B	31	-29.508	-0.716	-34.264	1.00	29.60	7	N
ATOM	1880	CA	VAL	B	31	-29.321	-2.091	-34.727	1.00	29.79	6	C
ATOM	1881	CB	VAL	B	31	-27.859	-2.398	-35.181	1.00	29.90	6	C
ATOM	1882	CG1	VAL	B	31	-26.890	-2.255	-34.045	1.00	29.99	6	C
ATOM	1883	CG2	VAL	B	31	-27.780	-3.784	-35.806	1.00	30.78	6	C
ATOM	1884	C	VAL	B	31	-29.858	-3.109	-33.711	1.00	29.27	6	C
ATOM	1885	O	VAL	B	31	-30.505	-4.081	-34.086	1.00	29.15	8	O
ATOM	1886	N	ASN	B	32	-29.637	-2.859	-32.424	1.00	29.19	7	N
ATOM	1887	CA	ASN	B	32	-30.106	-3.792	-31.407	1.00	29.31	6	C
ATOM	1888	CB	ASN	B	32	-29.550	-3.434	-30.021	1.00	28.38	6	C
ATOM	1889	CG	ASN	B	32	-28.034	-3.544	-29.955	1.00	28.07	6	C
ATOM	1890	OD1	ASN	B	32	-27.414	-4.173	-30.811	1.00	27.63	8	O
ATOM	1891	ND2	ASN	B	32	-27.429	-2.936	-28.930	1.00	26.72	7	N
ATOM	1892	C	ASN	B	32	-31.629	-3.892	-31.401	1.00	29.61	6	C
ATOM	1893	O	ASN	B	32	-32.175	-4.979	-31.303	1.00	29.49	8	O
ATOM	1894	N	ALA	B	33	-32.305	-2.752	-31.535	1.00	30.63	7	N
ATOM	1895	CA	ALA	B	33	-33.768	-2.699	-31.534	1.00	31.08	6	C
ATOM	1896	CB	ALA	B	33	-34.242	-1.259	-31.578	1.00	31.16	6	C
ATOM	1897	C	ALA	B	33	-34.396	-3.513	-32.676	1.00	31.77	6	C
ATOM	1898	O	ALA	B	33	-35.542	-3.989	-32.564	1.00	31.28	8	O
ATOM	1899	N	SER	B	34	-33.642	-3.696	-33.759	1.00	31.76	7	N
ATOM	1900	CA	SER	B	34	-34.128	-4.482	-34.889	1.00	32.48	6	C
ATOM	1901	CB	SER	B	34	-33.389	-4.092	-36.177	1.00	32.31	6	C
ATOM	1902	OG	SER	B	34	-32.074	-4.628	-36.186	1.00	31.09	8	O
ATOM	1903	C	SER	B	34	-33.992	-5.993	-34.655	1.00	33.19	6	C
ATOM	1904	O	SER	B	34	-34.454	-6.791	-35.479	1.00	33.37	8	O
ATOM	1905	N	LYS	B	35	-33.370	-6.368	-33.535	1.00	33.91	7	N
ATOM	1906	CA	LYS	B	35	-33.127	-7.769	-33.169	1.00	34.68	6	C
ATOM	1907	CB	LYS	B	35	-34.403	-8.404	-32.615	1.00	35.29	6	C
ATOM	1908	CG	LYS	B	35	-35.058	-7.602	-31.485	1.00	36.72	6	C
ATOM	1909	CD	LYS	B	35	-34.692	-8.140	-30.122	1.00	40.57	6	C
ATOM	1910	CE	LYS	B	35	-35.496	-7.457	-29.015	1.00	40.76	6	C
ATOM	1911	NZ	LYS	B	35	-36.831	-8.105	-28.810	1.00	42.80	7	N
ATOM	1912	C	LYS	B	35	-32.630	-8.572	-34.366	1.00	34.80	6	C
ATOM	1913	O	LYS	B	35	-33.317	-9.475	-34.844	1.00	34.35	8	O
ATOM	1914	N	PRO	B	36	-31.430	-8.253	-34.837	1.00	35.09	7	N
ATOM	1915	CA	PRO	B	36	-30.903	-8.840	-36.077	1.00	35.28	6	C
ATOM	1916	CB	PRO	B	36	-29.565	-8.116	-36.256	1.00	35.30	6	C
ATOM	1917	CG	PRO	B	36	-29.192	-7.714	-34.847	1.00	35.62	6	C
ATOM	1918	CD	PRO	B	36	-30.496	-7.276	-34.249	1.00	34.89	6	C
ATOM	1919	C	PRO	B	36	-30.705	-10.360	-36.077	1.00	35.65	6	C
ATOM	1920	O	PRO	B	36	-30.595	-10.922	-37.167	1.00	35.42	8	O
ATOM	1921	N	SER	B	37	-30.662	-11.017	-34.916	1.00	35.73	7	N
ATOM	1922	CA	SER	B	37	-30.474	-12.469	-34.915	1.00	36.28	6	C
ATOM	1923	CB	SER	B	37	-29.538	-12.929	-33.789	1.00	36.41	6	C
ATOM	1924	OG	SER	B	37	-30.183	-12.868	-32.533	1.00	35.91	8	O
ATOM	1925	C	SER	B	37	-31.789	-13.239	-34.869	1.00	36.95	6	C
ATOM	1926	O	SER	B	37	-31.803	-14.464	-34.989	1.00	37.58	8	O
ATOM	1927	N	GLU	B	38	-32.893	-12.524	-34.699	1.00	37.36	7	N
ATOM	1928	CA	GLU	B	38	-34.199	-13.161	-34.657	1.00	38.54	6	C
ATOM	1929	CB	GLU	B	38	-35.069	-12.541	-33.555	1.00	38.20	6	C

ATOM	1930	CG	GLU	B	38	-34.497	-12.752	-32.162	1.00	39.65	6	C
ATOM	1931	CD	GLU	B	38	-35.307	-12.080	-31.061	1.00	41.33	6	C
ATOM	1932	OE1	GLU	B	38	-36.512	-11.811	-31.263	1.00	41.59	8	O
ATOM	1933	OE2	GLU	B	38	-34.733	-11.822	-29.983	1.00	42.51	8	O
ATOM	1934	C	GLU	B	38	-34.866	-13.032	-36.018	1.00	38.82	6	C
ATOM	1935	O	GLU	B	38	-35.934	-12.459	-36.143	1.00	39.40	8	O
ATOM	1936	N	ARG	B	39	-34.213	-13.558	-37.043	1.00	39.56	7	N
ATOM	1937	CA	ARG	B	39	-34.746	-13.508	-38.395	1.00	40.06	6	C
ATOM	1938	CB	ARG	B	39	-33.852	-12.652	-39.288	1.00	39.74	6	C
ATOM	1939	CG	ARG	B	39	-33.605	-11.249	-38.760	1.00	38.73	6	C
ATOM	1940	CD	ARG	B	39	-34.740	-10.274	-39.009	1.00	36.27	6	C
ATOM	1941	NE	ARG	B	39	-34.464	-8.983	-38.391	1.00	34.61	7	N
ATOM	1942	CZ	ARG	B	39	-33.754	-8.019	-38.963	1.00	35.62	6	C
ATOM	1943	NH1	ARG	B	39	-33.256	-8.188	-40.186	1.00	34.56	7	N
ATOM	1944	NH2	ARG	B	39	-33.550	-6.876	-38.318	1.00	34.83	7	N
ATOM	1945	C	ARG	B	39	-34.796	-14.920	-38.945	1.00	40.94	6	C
ATOM	1946	O	ARG	B	39	-33.995	-15.768	-38.558	1.00	41.00	8	O
ATOM	1947	N	GLY	B	40	-35.742	-15.175	-39.843	1.00	41.86	7	N
ATOM	1948	CA	GLY	B	40	-35.849	-16.479	-40.464	1.00	42.75	6	C
ATOM	1949	C	GLY	B	40	-34.620	-16.743	-41.309	1.00	43.43	6	C
ATOM	1950	O	GLY	B	40	-33.996	-17.798	-41.210	1.00	44.09	8	O
ATOM	1951	N	LEU	B	41	-34.265	-15.773	-42.142	1.00	43.47	7	N
ATOM	1952	CA	LEU	B	41	-33.093	-15.910	-42.992	1.00	43.77	6	C
ATOM	1953	CB	LEU	B	41	-33.485	-15.855	-44.473	1.00	43.92	6	C
ATOM	1954	CG	LEU	B	41	-32.312	-15.792	-45.454	1.00	45.32	6	C
ATOM	1955	CD1	LEU	B	41	-31.384	-16.988	-45.271	1.00	46.78	6	C
ATOM	1956	CD2	LEU	B	41	-32.814	-15.720	-46.887	1.00	46.32	6	C
ATOM	1957	C	LEU	B	41	-32.071	-14.827	-42.675	1.00	43.35	6	C
ATOM	1958	O	LEU	B	41	-32.352	-13.638	-42.805	1.00	43.80	8	O
ATOM	1959	N	VAL	B	42	-30.890	-15.249	-42.242	1.00	42.64	7	N
ATOM	1960	CA	VAL	B	42	-29.816	-14.325	-41.922	1.00	42.03	6	C
ATOM	1961	CB	VAL	B	42	-29.002	-14.809	-40.696	1.00	42.22	6	C
ATOM	1962	CG1	VAL	B	42	-27.760	-13.961	-40.511	1.00	41.23	6	C
ATOM	1963	CG2	VAL	B	42	-29.853	-14.784	-39.434	1.00	42.53	6	C
ATOM	1964	C	VAL	B	42	-28.882	-14.217	-43.119	1.00	41.54	6	C
ATOM	1965	O	VAL	B	42	-28.497	-15.233	-43.691	1.00	41.43	8	O
ATOM	1966	N	ARG	B	43	-28.540	-12.994	-43.513	1.00	40.84	7	N
ATOM	1967	CA	ARG	B	43	-27.606	-12.783	-44.620	1.00	40.38	6	C
ATOM	1968	CB	ARG	B	43	-28.335	-12.301	-45.876	1.00	40.78	6	C
ATOM	1969	CG	ARG	B	43	-29.070	-13.411	-46.622	1.00	43.34	6	C
ATOM	1970	CD	ARG	B	43	-29.934	-12.922	-47.784	1.00	46.71	6	C
ATOM	1971	NE	ARG	B	43	-31.124	-12.220	-47.306	1.00	50.29	7	N
ATOM	1972	CZ	ARG	B	43	-31.778	-11.288	-47.995	1.00	51.44	6	C
ATOM	1973	NH1	ARG	B	43	-31.363	-10.939	-49.204	1.00	51.72	7	N
ATOM	1974	NH2	ARG	B	43	-32.850	-10.704	-47.469	1.00	52.75	7	N
ATOM	1975	C	ARG	B	43	-26.512	-11.803	-44.205	1.00	39.52	6	C
ATOM	1976	O	ARG	B	43	-26.335	-10.743	-44.809	1.00	38.70	8	O
ATOM	1977	N	GLN	B	44	-25.778	-12.187	-43.166	1.00	38.29	7	N
ATOM	1978	CA	GLN	B	44	-24.723	-11.364	-42.592	1.00	37.43	6	C
ATOM	1979	CB	GLN	B	44	-24.009	-12.154	-41.498	1.00	37.56	6	C
ATOM	1980	CG	GLN	B	44	-23.236	-11.321	-40.507	1.00	39.18	6	C
ATOM	1981	CD	GLN	B	44	-22.846	-12.139	-39.295	1.00	40.77	6	C
ATOM	1982	OE1	GLN	B	44	-23.595	-13.024	-38.892	1.00	41.02	8	O
ATOM	1983	NE2	GLN	B	44	-21.674	-11.863	-38.726	1.00	41.75	7	N
ATOM	1984	C	GLN	B	44	-23.715	-10.889	-43.635	1.00	36.43	6	C
ATOM	1985	O	GLN	B	44	-23.247	-9.750	-43.585	1.00	35.59	8	O
ATOM	1986	N	ALA	B	45	-23.390	-11.762	-44.585	1.00	35.69	7	N

```
ATOM   1987  CA  ALA B  45     -22.416 -11.419 -45.614  1.00 35.09   6       C
ATOM   1988  CB  ALA B  45     -22.193 -12.599 -46.567  1.00 35.40   6       C
ATOM   1989  C   ALA B  45     -22.774 -10.149 -46.395  1.00 34.46   6       C
ATOM   1990  O   ALA B  45     -21.879  -9.416 -46.816  1.00 34.15   8       O
ATOM   1991  N   GLU B  46     -24.068  -9.882 -46.575  1.00 33.79   7       N
ATOM   1992  CA  GLU B  46     -24.499  -8.700 -47.329  1.00 33.73   6       C
ATOM   1993  CB  GLU B  46     -25.999  -8.765 -47.662  1.00 33.80   6       C
ATOM   1994  CG  GLU B  46     -26.421  -9.857 -48.641  1.00 35.36   6       C
ATOM   1995  CD  GLU B  46     -25.928  -9.626 -50.060  1.00 37.74   6       C
ATOM   1996  OE1 GLU B  46     -25.631  -8.468 -50.428  1.00 37.79   8       O
ATOM   1997  OE2 GLU B  46     -25.832 -10.620 -50.814  1.00 39.71   8       O
ATOM   1998  C   GLU B  46     -24.220  -7.389 -46.596  1.00 33.38   6       C
ATOM   1999  O   GLU B  46     -24.357  -6.307 -47.177  1.00 32.62   8       O
ATOM   2000  N   ALA B  47     -23.864  -7.481 -45.314  1.00 32.85   7       N
ATOM   2001  CA  ALA B  47     -23.568  -6.290 -44.528  1.00 33.07   6       C
ATOM   2002  CB  ALA B  47     -24.142  -6.413 -43.113  1.00 32.94   6       C
ATOM   2003  C   ALA B  47     -22.073  -5.995 -44.471  1.00 33.26   6       C
ATOM   2004  O   ALA B  47     -21.660  -4.966 -43.941  1.00 32.82   8       O
ATOM   2005  N   GLU B  48     -21.265  -6.902 -45.007  1.00 33.94   7       N
ATOM   2006  CA  GLU B  48     -19.821  -6.705 -45.013  1.00 35.23   6       C
ATOM   2007  CB  GLU B  48     -19.107  -7.975 -45.482  1.00 35.50   6       C
ATOM   2008  CG  GLU B  48     -19.178  -9.119 -44.489  1.00 37.08   6       C
ATOM   2009  CD  GLU B  48     -18.470 -10.365 -44.981  1.00 39.75   6       C
ATOM   2010  OE1 GLU B  48     -17.398 -10.228 -45.611  1.00 42.17   8       O
ATOM   2011  OE2 GLU B  48     -18.981 -11.476 -44.734  1.00 39.89   8       O
ATOM   2012  C   GLU B  48     -19.413  -5.532 -45.899  1.00 35.92   6       C
ATOM   2013  O   GLU B  48     -19.998  -5.311 -46.953  1.00 35.83   8       O
ATOM   2014  N   ASP B  49     -18.406  -4.783 -45.464  1.00 36.95   7       N
ATOM   2015  CA  ASP B  49     -17.895  -3.667 -46.247  1.00 38.46   6       C
ATOM   2016  CB  ASP B  49     -18.668  -2.378 -45.949  1.00 38.28   6       C
ATOM   2017  CG  ASP B  49     -18.434  -1.306 -46.997  1.00 39.03   6       C
ATOM   2018  OD1 ASP B  49     -17.482  -1.460 -47.788  1.00 39.15   8       O
ATOM   2019  OD2 ASP B  49     -19.143  -0.282 -47.113  1.00 39.57   8       O
ATOM   2020  C   ASP B  49     -16.403  -3.482 -45.977  1.00 39.53   6       C
ATOM   2021  O   ASP B  49     -16.014  -2.811 -45.024  1.00 39.07   8       O
ATOM   2022  N   PRO B  50     -15.581  -4.100 -46.818  1.00 41.19   7       N
ATOM   2023  CA  PRO B  50     -14.114  -4.027 -46.710  1.00 42.47   6       C
ATOM   2024  CB  PRO B  50     -13.637  -4.691 -48.005  1.00 42.40   6       C
ATOM   2025  CG  PRO B  50     -14.750  -5.607 -48.395  1.00 42.26   6       C
ATOM   2026  CD  PRO B  50     -16.017  -4.935 -47.951  1.00 41.29   6       C
ATOM   2027  C   PRO B  50     -13.559  -2.605 -46.632  1.00 43.64   6       C
ATOM   2028  O   PRO B  50     -12.559  -2.378 -45.949  1.00 44.29   8       O
ATOM   2029  N   ALA B  51     -14.189  -1.663 -47.322  1.00 45.00   7       N
ATOM   2030  CA  ALA B  51     -13.724  -0.278 -47.311  1.00 45.79   6       C
ATOM   2031  CB  ALA B  51     -14.465   0.533 -48.344  1.00 46.07   6       C
ATOM   2032  C   ALA B  51     -13.897   0.348 -45.939  1.00 46.52   6       C
ATOM   2033  O   ALA B  51     -13.550   1.514 -45.726  1.00 46.62   8       O
ATOM   2034  N   CYS B  52     -14.424  -0.442 -45.008  1.00 46.79   7       N
ATOM   2035  CA  CYS B  52     -14.698   0.031 -43.662  1.00 47.44   6       C
ATOM   2036  CB  CYS B  52     -16.079  -0.430 -43.223  1.00 47.69   6       C
ATOM   2037  SG  CYS B  52     -17.315   0.800 -43.562  1.00 50.99  16       S
ATOM   2038  C   CYS B  52     -13.702  -0.439 -42.633  1.00 46.96   6       C
ATOM   2039  O   CYS B  52     -13.770  -0.026 -41.475  1.00 46.95   8       O
ATOM   2040  N   ILE B  53     -12.809  -1.334 -43.034  1.00 46.55   7       N
ATOM   2041  CA  ILE B  53     -11.809  -1.825 -42.107  1.00 46.54   6       C
ATOM   2042  CB  ILE B  53     -10.772  -2.698 -42.833  1.00 46.69   6       C
ATOM   2043  CG1 ILE B  53     -11.474  -3.857 -43.543  1.00 47.41   6       C
```

ATOM	2044	CD1	ILE	B	53	-10.540	-4.758	-44.349	1.00	47.75	6	C
ATOM	2045	CG2	ILE	B	53	-9.743	-3.236	-41.849	1.00	46.72	6	C
ATOM	2046	C	ILE	B	53	-11.170	-0.604	-41.462	1.00	46.15	6	C
ATOM	2047	O	ILE	B	53	-10.792	0.340	-42.151	1.00	46.05	8	O
ATOM	2048	N	PRO	B	54	-11.109	-0.600	-40.137	1.00	46.00	7	N
ATOM	2049	CA	PRO	B	54	-10.550	0.525	-39.384	1.00	45.97	6	C
ATOM	2050	CB	PRO	B	54	-10.600	0.025	-37.938	1.00	45.87	6	C
ATOM	2051	CG	PRO	B	54	-10.707	-1.466	-38.075	1.00	46.07	6	C
ATOM	2052	CD	PRO	B	54	-11.613	-1.657	-39.244	1.00	45.94	6	C
ATOM	2053	C	PRO	B	54	-9.114	0.854	-39.779	1.00	46.00	6	C
ATOM	2054	O	PRO	B	54	-8.362	-0.018	-40.220	1.00	46.11	8	O
ATOM	2055	N	ILE	B	55	-8.747	2.120	-39.637	1.00	45.93	7	N
ATOM	2056	CA	ILE	B	55	-7.383	2.536	-39.926	1.00	46.00	6	C
ATOM	2057	CB	ILE	B	55	-7.316	4.056	-40.160	1.00	46.03	6	C
ATOM	2058	CG1	ILE	B	55	-8.341	4.480	-41.213	1.00	46.99	6	C
ATOM	2059	CD1	ILE	B	55	-8.567	5.979	-41.276	1.00	47.02	6	C
ATOM	2060	CG2	ILE	B	55	-5.921	4.469	-40.603	1.00	46.63	6	C
ATOM	2061	C	ILE	B	55	-6.509	2.133	-38.742	1.00	45.49	6	C
ATOM	2062	O	ILE	B	55	-5.387	1.655	-38.921	1.00	45.69	8	O
ATOM	2063	N	PHE	B	56	-7.048	2.294	-37.536	1.00	44.71	7	N
ATOM	2064	CA	PHE	B	56	-6.321	1.969	-36.312	1.00	44.06	6	C
ATOM	2065	CB	PHE	B	56	-5.940	3.250	-35.561	1.00	44.46	6	C
ATOM	2066	CG	PHE	B	56	-5.109	4.209	-36.357	1.00	45.29	6	C
ATOM	2067	CD1	PHE	B	56	-5.662	5.375	-36.854	1.00	46.35	6	C
ATOM	2068	CE1	PHE	B	56	-4.893	6.274	-37.576	1.00	46.75	6	C
ATOM	2069	CZ	PHE	B	56	-3.557	6.008	-37.807	1.00	46.64	6	C
ATOM	2070	CE2	PHE	B	56	-2.993	4.850	-37.311	1.00	46.33	6	C
ATOM	2071	CD2	PHE	B	56	-3.766	3.958	-36.589	1.00	46.39	6	C
ATOM	2072	C	PHE	B	56	-7.125	1.112	-35.338	1.00	43.15	6	C
ATOM	2073	O	PHE	B	56	-8.349	1.247	-35.242	1.00	42.86	8	O
ATOM	2074	N	TRP	B	57	-6.422	0.240	-34.617	1.00	41.94	7	N
ATOM	2075	CA	TRP	B	57	-6.995	-0.506	-33.496	1.00	41.32	6	C
ATOM	2076	CB	TRP	B	57	-7.742	-1.778	-33.932	1.00	40.91	6	C
ATOM	2077	CG	TRP	B	57	-6.895	-2.795	-34.636	1.00	39.37	6	C
ATOM	2078	CD1	TRP	B	57	-6.168	-3.799	-34.069	1.00	39.14	6	C
ATOM	2079	NE1	TRP	B	57	-5.524	-4.528	-35.042	1.00	38.59	7	N
ATOM	2080	CE2	TRP	B	57	-5.840	-4.002	-36.268	1.00	39.25	6	C
ATOM	2081	CD2	TRP	B	57	-6.705	-2.912	-36.046	1.00	38.79	6	C
ATOM	2082	CE3	TRP	B	57	-7.175	-2.197	-37.153	1.00	38.60	6	C
ATOM	2083	CZ3	TRP	B	57	-6.779	-2.588	-38.420	1.00	39.95	6	C
ATOM	2084	CH2	TRP	B	57	-5.912	-3.673	-38.603	1.00	39.37	6	C
ATOM	2085	CZ2	TRP	B	57	-5.435	-4.390	-37.543	1.00	39.00	6	C
ATOM	2086	C	TRP	B	57	-5.890	-0.836	-32.493	1.00	41.24	6	C
ATOM	2087	O	TRP	B	57	-4.708	-0.772	-32.824	1.00	41.34	8	O
ATOM	2088	N	VAL	B	58	-6.277	-1.167	-31.267	1.00	40.90	7	N
ATOM	2089	CA	VAL	B	58	-5.322	-1.524	-30.229	1.00	40.95	6	C
ATOM	2090	CB	VAL	B	58	-5.938	-1.346	-28.827	1.00	40.92	6	C
ATOM	2091	CG1	VAL	B	58	-4.980	-1.810	-27.745	1.00	41.17	6	C
ATOM	2092	CG2	VAL	B	58	-6.335	0.112	-28.606	1.00	41.00	6	C
ATOM	2093	C	VAL	B	58	-4.852	-2.967	-30.424	1.00	40.88	6	C
ATOM	2094	O	VAL	B	58	-5.644	-3.906	-30.328	1.00	40.63	8	O
ATOM	2095	N	SER	B	59	-3.562	-3.137	-30.710	1.00	40.71	7	N
ATOM	2096	CA	SER	B	59	-3.004	-4.469	-30.963	1.00	40.82	6	C
ATOM	2097	CB	SER	B	59	-1.917	-4.400	-32.039	1.00	41.06	6	C
ATOM	2098	OG	SER	B	59	-1.090	-3.266	-31.856	1.00	41.44	8	O
ATOM	2099	C	SER	B	59	-2.473	-5.163	-29.708	1.00	40.52	6	C
ATOM	2100	O	SER	B	59	-2.444	-6.395	-29.631	1.00	40.41	8	O

ATOM	2101	N	LYS	B	60	-2.046	-4.362	-28.738	1.00	39.92	7	N
ATOM	2102	CA	LYS	B	60	-1.557	-4.854	-27.459	1.00	39.51	6	C
ATOM	2103	CB	LYS	B	60	-0.055	-5.160	-27.518	1.00	39.60	6	C
ATOM	2104	CG	LYS	B	60	0.448	-5.788	-28.809	1.00	40.15	6	C
ATOM	2105	CD	LYS	B	60	1.919	-6.195	-28.675	1.00	41.00	6	C
ATOM	2106	CE	LYS	B	60	2.458	-6.765	-29.982	1.00	41.88	6	C
ATOM	2107	NZ	LYS	B	60	3.869	-7.271	-29.822	1.00	42.94	7	N
ATOM	2108	C	LYS	B	60	-1.776	-3.763	-26.414	1.00	39.09	6	C
ATOM	2109	O	LYS	B	60	-1.808	-2.578	-26.745	1.00	38.83	8	O
ATOM	**2110**	**N**	**TRP**	**B**	**61**	**-1.929**	**-4.167**	**-25.161**	**1.00**	**38.91**	**7**	**N**
ATOM	**2111**	**CA**	**TRP**	**B**	**61**	**-2.053**	**-3.211**	**-24.063**	**1.00**	**39.28**	**6**	**C**
ATOM	**2112**	**CB**	**TRP**	**B**	**61**	**-3.510**	**-2.759**	**-23.878**	**1.00**	**38.78**	**6**	**C**
ATOM	**2113**	**CG**	**TRP**	**B**	**61**	**-4.472**	**-3.888**	**-23.641**	**1.00**	**37.70**	**6**	**C**
ATOM	**2114**	**CD1**	**TRP**	**B**	**61**	**-5.204**	**-4.554**	**-24.586**	**1.00**	**35.95**	**6**	**C**
ATOM	**2115**	**NE1**	**TRP**	**B**	**61**	**-5.973**	**-5.524**	**-23.992**	**1.00**	**36.01**	**7**	**N**
ATOM	**2116**	**CE2**	**TRP**	**B**	**61**	**-5.754**	**-5.499**	**-22.641**	**1.00**	**36.60**	**6**	**C**
ATOM	**2117**	**CD2**	**TRP**	**B**	**61**	**-4.814**	**-4.479**	**-22.385**	**1.00**	**36.92**	**6**	**C**
ATOM	**2118**	**CE3**	**TRP**	**B**	**61**	**-4.426**	**-4.248**	**-21.060**	**1.00**	**38.31**	**6**	**C**
ATOM	**2119**	**CZ3**	**TRP**	**B**	**61**	**-4.966**	**-5.036**	**-20.061**	**1.00**	**37.79**	**6**	**C**
ATOM	**2120**	**CH2**	**TRP**	**B**	**61**	**-5.893**	**-6.038**	**-20.351**	**1.00**	**38.23**	**6**	**C**
ATOM	**2121**	**CZ2**	**TRP**	**B**	**61**	**-6.298**	**-6.286**	**-21.633**	**1.00**	**36.55**	**6**	**C**
ATOM	**2122**	**C**	**TRP**	**B**	**61**	**-1.501**	**-3.776**	**-22.758**	**1.00**	**39.88**	**6**	**C**
ATOM	**2123**	**O**	**TRP**	**B**	**61**	**-1.449**	**-4.993**	**-22.566**	**1.00**	**40.11**	**8**	**O**
ATOM	2124	N	VAL	B	62	-1.084	-2.877	-21.871	1.00	40.59	7	N
ATOM	2125	CA	VAL	B	62	-0.560	-3.245	-20.563	1.00	41.45	6	C
ATOM	2126	CB	VAL	B	62	0.977	-3.140	-20.510	1.00	41.29	6	C
ATOM	2127	CG1	VAL	B	62	1.480	-3.465	-19.115	1.00	41.94	6	C
ATOM	2128	CG2	VAL	B	62	1.615	-4.067	-21.519	1.00	41.97	6	C
ATOM	2129	C	VAL	B	62	-1.156	-2.297	-19.524	1.00	41.74	6	C
ATOM	2130	O	VAL	B	62	-0.977	-1.081	-19.612	1.00	41.59	8	O
ATOM	2131	N	ASP	B	63	-1.863	-2.861	-18.551	1.00	42.53	7	N
ATOM	2132	CA	ASP	B	63	-2.522	-2.083	-17.512	1.00	43.68	6	C
ATOM	2133	CB	ASP	B	63	-3.831	-2.755	-17.093	1.00	43.55	6	C
ATOM	2134	CG	ASP	B	63	-4.525	-2.030	-15.956	1.00	43.95	6	C
ATOM	2135	OD1	ASP	B	63	-4.019	-0.969	-15.526	1.00	43.59	8	O
ATOM	2136	OD2	ASP	B	63	-5.576	-2.450	-15.421	1.00	43.71	8	O
ATOM	2137	C	ASP	B	63	-1.632	-1.893	-16.287	1.00	44.67	6	C
ATOM	2138	O	ASP	B	63	-1.605	-2.734	-15.387	1.00	44.34	8	O
ATOM	2139	N	TYR	B	64	-0.905	-0.784	-16.267	1.00	46.03	7	N
ATOM	2140	CA	TYR	B	64	-0.073	-0.445	-15.120	1.00	47.40	6	C
ATOM	2141	CB	TYR	B	64	1.380	-0.222	-15.540	1.00	47.82	6	C
ATOM	2142	CG	TYR	B	64	2.167	-1.506	-15.659	1.00	50.03	6	C
ATOM	2143	CD1	TYR	B	64	3.478	-1.504	-16.112	1.00	51.96	6	C
ATOM	2144	CE1	TYR	B	64	4.197	-2.688	-16.220	1.00	53.33	6	C
ATOM	2145	CZ	TYR	B	64	3.602	-3.884	-15.867	1.00	53.34	6	C
ATOM	2146	OH	TYR	B	64	4.307	-5.062	-15.968	1.00	54.77	8	O
ATOM	2147	CE2	TYR	B	64	2.305	-3.907	-15.412	1.00	52.75	6	C
ATOM	2148	CD2	TYR	B	64	1.596	-2.725	-15.311	1.00	51.72	6	C
ATOM	2149	C	TYR	B	64	-0.642	0.802	-14.462	1.00	47.57	6	C
ATOM	2150	O	TYR	B	64	0.101	1.633	-13.947	1.00	47.39	8	O
ATOM	2151	N	SER	B	65	-1.968	0.923	-14.501	1.00	47.80	7	N
ATOM	2152	CA	SER	B	65	-2.670	2.066	-13.918	1.00	48.23	6	C
ATOM	2153	CB	SER	B	65	-4.162	2.023	-14.277	1.00	48.08	6	C
ATOM	2154	OG	SER	B	65	-4.788	0.876	-13.726	1.00	47.43	8	O
ATOM	2155	C	SER	B	65	-2.492	2.106	-12.404	1.00	48.73	6	C
ATOM	2156	O	SER	B	65	-2.846	3.088	-11.749	1.00	48.86	8	O
ATOM	2157	N	ASP	B	66	-1.946	1.021	-11.864	1.00	49.45	7	N

ATOM	2158	CA	ASP	B	66	-1.649	0.888	-10.442	1.00	50.03	6	C
ATOM	2159	CB	ASP	B	66	-0.936	-0.444	-10.197	1.00	50.24	6	C
ATOM	2160	CG	ASP	B	66	-1.298	-1.071	-8.871	1.00	51.55	6	C
ATOM	2161	OD1	ASP	B	66	-1.291	-0.358	-7.843	1.00	53.29	8	O
ATOM	2162	OD2	ASP	B	66	-1.598	-2.281	-8.761	1.00	53.05	8	O
ATOM	2163	C	ASP	B	66	-0.740	2.020	-9.980	1.00	49.99	6	C
ATOM	2164	O	ASP	B	66	-0.898	2.552	-8.879	1.00	50.31	8	O
ATOM	2165	N	LYS	B	67	0.217	2.388	-10.824	1.00	49.83	7	N
ATOM	2166	CA	LYS	B	67	1.187	3.408	-10.445	1.00	49.88	6	C
ATOM	2167	CB	LYS	B	67	2.486	2.744	-9.968	1.00	50.16	6	C
ATOM	2168	CG	LYS	B	67	2.292	1.606	-8.970	1.00	50.67	6	C
ATOM	2169	CD	LYS	B	67	3.628	1.115	-8.427	1.00	52.01	6	C
ATOM	2170	CE	LYS	B	67	3.426	-0.029	-7.441	1.00	52.48	6	C
ATOM	2171	NZ	LYS	B	67	4.654	-0.315	-6.657	1.00	52.33	7	N
ATOM	2172	C	LYS	B	67	1.521	4.402	-11.555	1.00	49.63	6	C
ATOM	2173	O	LYS	B	67	1.918	5.534	-11.273	1.00	49.74	8	O
ATOM	2174	N	TYR	B	68	1.367	3.988	-12.811	1.00	49.08	7	N
ATOM	2175	CA	TYR	B	68	1.777	4.841	-13.924	1.00	48.69	6	C
ATOM	2176	CB	TYR	B	68	2.941	4.188	-14.664	1.00	48.97	6	C
ATOM	2177	CG	TYR	B	68	4.094	3.857	-13.750	1.00	50.19	6	C
ATOM	2178	CD1	TYR	B	68	4.546	2.553	-13.608	1.00	51.24	6	C
ATOM	2179	CE1	TYR	B	68	5.603	2.252	-12.761	1.00	52.27	6	C
ATOM	2180	CZ	TYR	B	68	6.207	3.265	-12.043	1.00	52.23	6	C
ATOM	2181	OH	TYR	B	68	7.254	2.982	-11.197	1.00	53.59	8	O
ATOM	2182	CE2	TYR	B	68	5.771	4.563	-12.167	1.00	51.84	6	C
ATOM	2183	CD2	TYR	B	68	4.719	4.852	-13.013	1.00	51.41	6	C
ATOM	2184	C	TYR	B	68	0.673	5.200	-14.911	1.00	48.02	6	C
ATOM	2185	O	TYR	B	68	0.413	6.376	-15.160	1.00	48.00	8	O
ATOM	2186	N	GLY	B	69	0.050	4.178	-15.490	1.00	47.28	7	N
ATOM	2187	CA	GLY	B	69	-0.998	4.379	-16.475	1.00	46.31	6	C
ATOM	2188	C	GLY	B	69	-1.173	3.145	-17.341	1.00	45.70	6	C
ATOM	2189	O	GLY	B	69	-0.731	2.056	-16.975	1.00	45.54	8	O
ATOM	2190	N	LEU	B	70	-1.823	3.308	-18.487	1.00	44.96	7	N
ATOM	2191	CA	LEU	B	70	-2.035	2.182	-19.389	1.00	44.33	6	C
ATOM	2192	CB	LEU	B	70	-3.526	1.973	-19.674	1.00	44.42	6	C
ATOM	2193	CG	LEU	B	70	-3.858	0.689	-20.448	1.00	44.65	6	C
ATOM	2194	CD1	LEU	B	70	-5.027	-0.062	-19.821	1.00	44.25	6	C
ATOM	2195	CD2	LEU	B	70	-4.105	0.990	-21.921	1.00	44.67	6	C
ATOM	2196	C	LEU	B	70	-1.257	2.377	-20.679	1.00	43.72	6	C
ATOM	2197	O	LEU	B	70	-1.379	3.405	-21.336	1.00	43.83	8	O
ATOM	2198	N	GLY	B	71	-0.443	1.387	-21.022	1.00	43.34	7	N
ATOM	2199	CA	GLY	B	71	0.350	1.426	-22.232	1.00	42.55	6	C
ATOM	2200	C	GLY	B	71	-0.299	0.531	-23.260	1.00	42.19	6	C
ATOM	2201	O	GLY	B	71	-0.919	-0.468	-22.919	1.00	41.85	8	O
ATOM	2202	N	TYR	B	72	-0.142	0.878	-24.526	1.00	42.15	7	N
ATOM	2203	CA	TYR	B	72	-0.806	0.131	-25.574	1.00	42.11	6	C
ATOM	2204	CB	TYR	B	72	-2.250	0.641	-25.732	1.00	41.72	6	C
ATOM	2205	CG	TYR	B	72	-2.334	2.099	-26.140	1.00	40.71	6	C
ATOM	2206	CD1	TYR	B	72	-2.322	2.467	-27.481	1.00	39.92	6	C
ATOM	2207	CE1	TYR	B	72	-2.383	3.792	-27.860	1.00	38.64	6	C
ATOM	2208	CZ	TYR	B	72	-2.457	4.776	-26.895	1.00	37.83	6	C
ATOM	2209	OH	TYR	B	72	-2.527	6.092	-27.283	1.00	37.64	8	O
ATOM	2210	CE2	TYR	B	72	-2.473	4.444	-25.562	1.00	37.39	6	C
ATOM	2211	CD2	TYR	B	72	-2.409	3.112	-25.188	1.00	40.00	6	C
ATOM	2212	C	TYR	B	72	-0.069	0.321	-26.877	1.00	42.59	6	C
ATOM	2213	O	TYR	B	72	0.690	1.276	-27.039	1.00	42.25	8	O
ATOM	2214	N	GLN	B	73	-0.296	-0.598	-27.804	1.00	43.00	7	N

ATOM	2215	CA	GLN	B	73	0.271	-0.479	-29.130	1.00	43.94	6	C
ATOM	2216	CB	GLN	B	73	1.159	-1.686	-29.457	1.00	43.99	6	C
ATOM	2217	CG	GLN	B	73	1.751	-1.635	-30.868	1.00	44.38	6	C
ATOM	2218	CD	GLN	B	73	2.136	-3.000	-31.409	1.00	45.38	6	C
ATOM	2219	OE1	GLN	B	73	1.291	-3.892	-31.519	1.00	45.59	8	O
ATOM	2220	NE2	GLN	B	73	3.407	-3.162	-31.764	1.00	44.82	7	N
ATOM	2221	C	GLN	B	73	-0.870	-0.395	-30.130	1.00	44.38	6	C
ATOM	2222	O	GLN	B	73	-1.900	-1.046	-29.961	1.00	44.15	8	O
ATOM	2223	N	LEU	B	74	-0.700	0.435	-31.151	1.00	45.26	7	N
ATOM	2224	CA	LEU	B	74	-1.666	0.500	-32.233	1.00	46.12	6	C
ATOM	2225	CB	LEU	B	74	-1.819	1.928	-32.749	1.00	46.00	6	C
ATOM	2226	CG	LEU	B	74	-2.463	2.946	-31.806	1.00	45.43	6	C
ATOM	2227	CD1	LEU	B	74	-2.767	4.221	-32.563	1.00	45.01	6	C
ATOM	2228	CD2	LEU	B	74	-3.730	2.382	-31.184	1.00	45.18	6	C
ATOM	2229	C	LEU	B	74	-1.137	-0.407	-33.335	1.00	47.07	6	C
ATOM	2230	O	LEU	B	74	0.076	-0.536	-33.502	1.00	47.34	8	O
ATOM	2231	N	CYS	B	75	-2.036	-1.048	-34.074	1.00	47.91	7	N
ATOM	2232	CA	CYS	B	75	-1.637	-1.956	-35.149	1.00	48.87	6	C
ATOM	2233	CB	CYS	B	75	-2.858	-2.361	-35.967	1.00	48.61	6	C
ATOM	2234	SG	CYS	B	75	-3.706	-0.962	-36.722	1.00	49.27	16	S
ATOM	2235	C	CYS	B	75	-0.617	-1.289	-36.066	1.00	49.39	6	C
ATOM	2236	O	CYS	B	75	-0.059	-1.916	-36.966	1.00	49.37	8	O
ATOM	2237	N	ASP	B	76	-0.396	-0.003	-35.820	1.00	50.09	7	N
ATOM	2238	CA	ASP	B	76	0.507	0.828	-36.602	1.00	50.55	6	C
ATOM	2239	CB	ASP	B	76	0.195	2.297	-36.309	1.00	50.73	6	C
ATOM	2240	CG	ASP	B	76	0.719	3.229	-37.378	1.00	51.81	6	C
ATOM	2241	OD1	ASP	B	76	0.891	2.775	-38.531	1.00	53.10	8	O
ATOM	2242	OD2	ASP	B	76	0.976	4.430	-37.163	1.00	51.77	8	O
ATOM	2243	C	ASP	B	76	1.954	0.560	-36.233	1.00	50.56	6	C
ATOM	2244	O	ASP	B	76	2.876	0.983	-36.936	1.00	50.71	8	O
ATOM	2245	N	ASN	B	77	2.147	-0.147	-35.124	1.00	50.40	7	N
ATOM	2246	CA	ASN	B	77	3.473	-0.393	-34.582	1.00	50.27	6	C
ATOM	2247	CB	ASN	B	77	4.505	-0.595	-35.690	1.00	50.38	6	C
ATOM	2248	CG	ASN	B	77	4.327	-1.907	-36.410	1.00	51.17	6	C
ATOM	2249	OD1	ASN	B	77	4.226	-2.962	-35.783	1.00	52.10	8	O
ATOM	2250	ND2	ASN	B	77	4.283	-1.854	-37.736	1.00	51.93	7	N
ATOM	2251	C	ASN	B	77	3.849	0.784	-33.701	1.00	49.87	6	C
ATOM	2252	O	ASN	B	77	4.838	0.746	-32.966	1.00	49.97	8	O
ATOM	2253	N	SER	B	78	3.051	1.843	-33.795	1.00	49.30	7	N
ATOM	2254	CA	SER	B	78	3.225	3.000	-32.939	1.00	48.55	6	C
ATOM	2255	CB	SER	B	78	2.419	4.187	-33.464	1.00	48.82	6	C
ATOM	2256	OG	SER	B	78	1.025	3.928	-33.415	1.00	49.14	8	O
ATOM	2257	C	SER	B	78	2.729	2.588	-31.564	1.00	47.91	6	C
ATOM	2258	O	SER	B	78	1.936	1.657	-31.445	1.00	47.73	8	O
ATOM	2259	N	VAL	B	79	3.205	3.264	-30.526	1.00	47.11	7	N
ATOM	2260	CA	VAL	B	79	2.797	2.940	-29.165	1.00	46.32	6	C
ATOM	2261	CB	VAL	B	79	3.923	2.246	-28.365	1.00	46.43	6	C
ATOM	2262	CG1	VAL	B	79	4.309	0.926	-29.008	1.00	46.40	6	C
ATOM	2263	CG2	VAL	B	79	5.137	3.155	-28.243	1.00	46.59	6	C
ATOM	2264	C	VAL	B	79	2.361	4.197	-28.440	1.00	45.82	6	C
ATOM	2265	O	VAL	B	79	2.638	5.315	-28.885	1.00	45.91	8	O
ATOM	2266	N	GLY	B	80	1.674	4.017	-27.323	1.00	45.07	7	N
ATOM	2267	CA	GLY	B	80	1.177	5.149	-26.574	1.00	44.81	6	C
ATOM	2268	C	GLY	B	80	0.838	4.787	-25.148	1.00	44.62	6	C
ATOM	2269	O	GLY	B	80	0.876	3.620	-24.755	1.00	44.13	8	O
ATOM	2270	N	VAL	B	81	0.491	5.802	-24.370	1.00	44.84	7	N
ATOM	2271	CA	VAL	B	81	0.161	5.594	-22.976	1.00	45.14	6	C

```
ATOM   2272  CB  VAL B  81       1.441   5.569 -22.116  1.00 45.19      6       C
ATOM   2273  CG1 VAL B  81       2.311   6.762 -22.452  1.00 45.41      6       C
ATOM   2274  CG2 VAL B  81       1.112   5.535 -20.626  1.00 45.15      6       C
ATOM   2275  C   VAL B  81      -0.759   6.695 -22.480  1.00 45.31      6       C
ATOM   2276  O   VAL B  81      -0.682   7.842 -22.926  1.00 45.36      8       O
ATOM   2277  N   LEU B  82      -1.653   6.327 -21.573  1.00 45.75      7       N
ATOM   2278  CA  LEU B  82      -2.521   7.290 -20.919  1.00 46.32      6       C
ATOM   2279  CB  LEU B  82      -3.994   6.893 -21.052  1.00 46.22      6       C
ATOM   2280  CG  LEU B  82      -4.986   7.694 -20.203  1.00 46.72      6       C
ATOM   2281  CD1 LEU B  82      -4.728   9.186 -20.324  1.00 46.40      6       C
ATOM   2282  CD2 LEU B  82      -6.428   7.359 -20.587  1.00 47.47      6       C
ATOM   2283  C   LEU B  82      -2.087   7.267 -19.469  1.00 46.70      6       C
ATOM   2284  O   LEU B  82      -2.391   6.323 -18.737  1.00 46.54      8       O
ATOM   2285  N   PHE B  83      -1.338   8.290 -19.071  1.00 47.21      7       N
ATOM   2286  CA  PHE B  83      -0.816   8.379 -17.713  1.00 48.06      6       C
ATOM   2287  CB  PHE B  83       0.311   9.411 -17.644  1.00 47.70      6       C
ATOM   2288  CG  PHE B  83       1.547   9.006 -18.400  1.00 47.11      6       C
ATOM   2289  CD1 PHE B  83       1.882   9.627 -19.590  1.00 46.02      6       C
ATOM   2290  CE1 PHE B  83       3.021   9.251 -20.284  1.00 45.84      6       C
ATOM   2291  CZ  PHE B  83       3.835   8.251 -19.788  1.00 45.40      6       C
ATOM   2292  CE2 PHE B  83       3.511   7.626 -18.603  1.00 45.30      6       C
ATOM   2293  CD2 PHE B  83       2.375   8.002 -17.915  1.00 46.06      6       C
ATOM   2294  C   PHE B  83      -1.907   8.711 -16.704  1.00 48.88      6       C
ATOM   2295  O   PHE B  83      -2.950   9.256 -17.065  1.00 48.97      8       O
ATOM   2296  N   ASN B  84      -1.650   8.386 -15.438  1.00 49.94      7       N
ATOM   2297  CA  ASN B  84      -2.614   8.594 -14.361  1.00 50.92      6       C
ATOM   2298  CB  ASN B  84      -2.119   7.949 -13.068  1.00 50.84      6       C
ATOM   2299  CG  ASN B  84      -2.358   6.457 -13.036  1.00 51.37      6       C
ATOM   2300  OD1 ASN B  84      -2.907   5.882 -13.979  1.00 51.80      8       O
ATOM   2301  ND2 ASN B  84      -1.947   5.816 -11.946  1.00 51.25      7       N
ATOM   2302  C   ASN B  84      -3.010  10.044 -14.095  1.00 51.66      6       C
ATOM   2303  O   ASN B  84      -3.951  10.302 -13.348  1.00 51.79      8       O
ATOM   2304  N   ASN B  85      -2.290  10.990 -14.688  1.00 52.45      7       N
ATOM   2305  CA  ASN B  85      -2.637  12.397 -14.524  1.00 53.40      6       C
ATOM   2306  CB  ASN B  85      -1.383  13.253 -14.343  1.00 53.43      6       C
ATOM   2307  CG  ASN B  85      -0.268  12.849 -15.281  1.00 54.35      6       C
ATOM   2308  OD1 ASN B  85      -0.458  12.781 -16.495  1.00 54.69      8       O
ATOM   2309  ND2 ASN B  85       0.905  12.563 -14.721  1.00 55.14      7       N
ATOM   2310  C   ASN B  85      -3.463  12.901 -15.701  1.00 53.75      6       C
ATOM   2311  O   ASN B  85      -3.652  14.105 -15.872  1.00 53.84      8       O
ATOM   2312  N   SER B  86      -3.938  11.964 -16.517  1.00 54.18      7       N
ATOM   2313  CA  SER B  86      -4.770  12.285 -17.672  1.00 54.59      6       C
ATOM   2314  CB  SER B  86      -5.810  13.348 -17.308  1.00 54.75      6       C
ATOM   2315  OG  SER B  86      -6.539  12.975 -16.148  1.00 55.35      8       O
ATOM   2316  C   SER B  86      -3.972  12.713 -18.911  1.00 54.75      6       C
ATOM   2317  O   SER B  86      -4.550  12.927 -19.977  1.00 54.87      8       O
ATOM   2318  N   THR B  87      -2.654  12.846 -18.782  1.00 54.78      7       N
ATOM   2319  CA  THR B  87      -1.838  13.224 -19.935  1.00 54.86      6       C
ATOM   2320  CB  THR B  87      -0.513  13.884 -19.510  1.00 54.79      6       C
ATOM   2321  OG1 THR B  87       0.303  12.929 -18.821  1.00 54.67      8       O
ATOM   2322  CG2 THR B  87      -0.761  14.972 -18.477  1.00 55.12      6       C
ATOM   2323  C   THR B  87      -1.548  12.008 -20.803  1.00 54.96      6       C
ATOM   2324  O   THR B  87      -1.619  10.873 -20.338  1.00 54.54      8       O
ATOM   2325  N   ARG B  88      -1.210  12.257 -22.063  1.00 55.37      7       N
ATOM   2326  CA  ARG B  88      -0.937  11.181 -23.003  1.00 55.98      6       C
ATOM   2327  CB  ARG B  88      -2.129  10.996 -23.944  1.00 56.11      6       C
ATOM   2328  CG  ARG B  88      -3.465  11.001 -23.216  1.00 56.90      6       C
```

```
ATOM   2329  CD  ARG B  88      -4.642  11.404 -24.076  1.00 58.32      6       C
ATOM   2330  NE  ARG B  88      -5.564  12.295 -23.375  1.00 59.85      7       N
ATOM   2331  CZ  ARG B  88      -6.502  11.895 -22.528  1.00 60.67      6       C
ATOM   2332  NH1 ARG B  88      -6.657  10.607 -22.259  1.00 61.31      7       N
ATOM   2333  NH2 ARG B  88      -7.288  12.785 -21.944  1.00 61.21      7       N
ATOM   2334  C   ARG B  88       0.338  11.438 -23.798  1.00 56.15      6       C
ATOM   2335  O   ARG B  88       0.687  12.585 -24.083  1.00 56.09      8       O
ATOM   2336  N   LEU B  89       1.030  10.359 -24.146  1.00 56.45      7       N
ATOM   2337  CA  LEU B  89       2.266  10.442 -24.910  1.00 56.73      6       C
ATOM   2338  CB  LEU B  89       3.468  10.266 -23.986  1.00 56.69      6       C
ATOM   2339  CG  LEU B  89       4.845  10.423 -24.630  1.00 56.69      6       C
ATOM   2340  CD1 LEU B  89       4.954  11.755 -25.358  1.00 56.62      6       C
ATOM   2341  CD2 LEU B  89       5.934  10.287 -23.578  1.00 56.68      6       C
ATOM   2342  C   LEU B  89       2.275   9.372 -25.996  1.00 56.98      6       C
ATOM   2343  O   LEU B  89       2.106   8.188 -25.711  1.00 56.74      8       O
ATOM   2344  N   ILE B  90       2.468   9.798 -27.240  1.00 57.42      7       N
ATOM   2345  CA  ILE B  90       2.462   8.887 -28.380  1.00 58.03      6       C
ATOM   2346  CB  ILE B  90       1.402   9.332 -29.412  1.00 57.99      6       C
ATOM   2347  CG1 ILE B  90       0.034   8.739 -29.072  1.00 58.00      6       C
ATOM   2348  CD1 ILE B  90      -0.523   9.185 -27.745  1.00 58.27      6       C
ATOM   2349  CG2 ILE B  90       1.803   8.899 -30.809  1.00 57.79      6       C
ATOM   2350  C   ILE B  90       3.825   8.778 -29.064  1.00 58.55      6       C
ATOM   2351  O   ILE B  90       4.454   9.788 -29.377  1.00 58.58      8       O
ATOM   2352  N   LEU B  91       4.268   7.546 -29.298  1.00 59.14      7       N
ATOM   2353  CA  LEU B  91       5.530   7.296 -29.985  1.00 59.96      6       C
ATOM   2354  CB  LEU B  91       6.424   6.379 -29.146  1.00 59.95      6       C
ATOM   2355  CG  LEU B  91       7.784   5.982 -29.726  1.00 60.22      6       C
ATOM   2356  CD1 LEU B  91       8.661   7.203 -29.972  1.00 60.08      6       C
ATOM   2357  CD2 LEU B  91       8.488   4.989 -28.812  1.00 60.59      6       C
ATOM   2358  C   LEU B  91       5.280   6.681 -31.363  1.00 60.42      6       C
ATOM   2359  O   LEU B  91       4.936   5.505 -31.468  1.00 60.57      8       O
ATOM   2360  N   TYR B  92       5.461   7.483 -32.411  1.00 61.14      7       N
ATOM   2361  CA  TYR B  92       5.237   7.050 -33.797  1.00 61.87      6       C
ATOM   2362  CB  TYR B  92       5.565   8.187 -34.769  1.00 61.89      6       C
ATOM   2363  CG  TYR B  92       4.556   9.313 -34.755  1.00 62.35      6       C
ATOM   2364  CD1 TYR B  92       4.621  10.320 -33.799  1.00 62.74      6       C
ATOM   2365  CE1 TYR B  92       3.698  11.351 -33.781  1.00 63.08      6       C
ATOM   2366  CZ  TYR B  92       2.694  11.382 -34.728  1.00 63.28      6       C
ATOM   2367  OH  TYR B  92       1.772  12.407 -34.716  1.00 63.24      8       O
ATOM   2368  CE2 TYR B  92       2.611  10.393 -35.689  1.00 62.82      6       C
ATOM   2369  CD2 TYR B  92       3.535   9.368 -35.696  1.00 62.47      6       C
ATOM   2370  C   TYR B  92       5.998   5.780 -34.198  1.00 62.32      6       C
ATOM   2371  O   TYR B  92       6.924   5.354 -33.507  1.00 62.29      8       O
ATOM   2372  N   ASN B  93       5.609   5.185 -35.325  1.00 62.98      7       N
ATOM   2373  CA  ASN B  93       6.243   3.949 -35.790  1.00 63.67      6       C
ATOM   2374  CB  ASN B  93       5.471   3.297 -36.952  1.00 63.71      6       C
ATOM   2375  CG  ASN B  93       5.126   4.275 -38.067  1.00 63.98      6       C
ATOM   2376  OD1 ASN B  93       5.878   5.205 -38.360  1.00 64.09      8       O
ATOM   2377  ND2 ASN B  93       3.982   4.054 -38.705  1.00 64.30      7       N
ATOM   2378  C   ASN B  93       7.736   4.084 -36.106  1.00 64.13      6       C
ATOM   2379  O   ASN B  93       8.316   3.243 -36.790  1.00 64.16      8       O
ATOM   2380  N   ASP B  94       8.342   5.156 -35.604  1.00 64.70      7       N
ATOM   2381  CA  ASP B  94       9.783   5.361 -35.701  1.00 65.19      6       C
ATOM   2382  CB  ASP B  94      10.155   6.394 -36.779  1.00 65.18      6       C
ATOM   2383  CG  ASP B  94       9.643   7.798 -36.473  1.00 65.18      6       C
ATOM   2384  OD1 ASP B  94       9.338   8.103 -35.304  1.00 65.04      8       O
ATOM   2385  OD2 ASP B  94       9.524   8.677 -37.352  1.00 65.48      8       O
```

ATOM	2386	C	ASP	B	94	10.287	5.771	-34.321	1.00	65.50	6	C
ATOM	2387	O	ASP	B	94	10.165	6.927	-33.925	1.00	65.67	8	O
ATOM	2388	N	GLY	B	95	10.829	4.809	-33.581	1.00	65.83	7	N
ATOM	2389	CA	GLY	B	95	11.301	5.041	-32.226	1.00	66.28	6	C
ATOM	2390	C	GLY	B	95	11.863	6.420	-31.919	1.00	66.59	6	C
ATOM	2391	O	GLY	B	95	12.755	6.552	-31.080	1.00	66.62	8	O
ATOM	2392	N	ASP	B	96	11.338	7.450	-32.578	1.00	66.88	7	N
ATOM	2393	CA	ASP	B	96	11.809	8.813	-32.356	1.00	67.18	6	C
ATOM	2394	CB	ASP	B	96	12.768	9.236	-33.474	1.00	67.27	6	C
ATOM	2395	CG	ASP	B	96	13.823	10.219	-32.997	1.00	67.45	6	C
ATOM	2396	OD1	ASP	B	96	13.594	10.896	-31.971	1.00	67.40	8	O
ATOM	2397	OD2	ASP	B	96	14.916	10.377	-33.581	1.00	68.03	8	O
ATOM	2398	C	ASP	B	96	10.672	9.835	-32.208	1.00	67.32	6	C
ATOM	2399	O	ASP	B	96	10.487	10.406	-31.134	1.00	67.40	8	O
ATOM	2400	N	SER	B	97	9.915	10.061	-33.281	1.00	67.42	7	N
ATOM	2401	CA	SER	B	97	8.832	11.053	-33.275	1.00	67.54	6	C
ATOM	2402	CB	SER	B	97	8.043	10.995	-34.584	1.00	67.55	6	C
ATOM	2403	OG	SER	B	97	8.855	11.364	-35.686	1.00	67.59	8	O
ATOM	2404	C	SER	B	97	7.880	10.931	-32.080	1.00	67.68	6	C
ATOM	2405	O	SER	B	97	7.582	9.827	-31.622	1.00	67.61	8	O
ATOM	2406	N	LEU	B	98	7.400	12.072	-31.586	1.00	67.81	7	N
ATOM	2407	CA	LEU	B	98	6.516	12.089	-30.422	1.00	68.00	6	C
ATOM	2408	CB	LEU	B	98	7.319	12.318	-29.140	1.00	67.93	6	C
ATOM	2409	CG	LEU	B	98	8.190	11.213	-28.554	1.00	67.95	6	C
ATOM	2410	CD1	LEU	B	98	9.010	11.789	-27.417	1.00	67.95	6	C
ATOM	2411	CD2	LEU	B	98	7.352	10.048	-28.070	1.00	67.97	6	C
ATOM	2412	C	LEU	B	98	5.420	13.146	-30.470	1.00	68.19	6	C
ATOM	2413	O	LEU	B	98	5.580	14.210	-31.069	1.00	68.13	8	O
ATOM	2414	N	GLN	B	99	4.309	12.832	-29.812	1.00	68.43	7	N
ATOM	2415	CA	GLN	B	99	3.202	13.759	-29.639	1.00	68.66	6	C
ATOM	2416	CB	GLN	B	99	2.012	13.382	-30.521	1.00	68.63	6	C
ATOM	2417	CG	GLN	B	99	0.804	14.293	-30.335	1.00	68.59	6	C
ATOM	2418	CD	GLN	B	99	-0.424	13.810	-31.085	1.00	68.71	6	C
ATOM	2419	OE1	GLN	B	99	-1.170	12.968	-30.587	1.00	68.60	8	O
ATOM	2420	NE2	GLN	B	99	-0.641	14.347	-32.278	1.00	68.61	7	N
ATOM	2421	C	GLN	B	99	2.802	13.701	-28.170	1.00	68.91	6	C
ATOM	2422	O	GLN	B	99	2.559	12.619	-27.634	1.00	68.84	8	O
ATOM	2423	N	TYR	B	100	2.757	14.858	-27.517	1.00	69.20	7	N
ATOM	2424	CA	TYR	B	100	2.380	14.927	-26.109	1.00	69.56	6	C
ATOM	2425	CB	TYR	B	100	3.503	15.557	-25.282	1.00	69.32	6	C
ATOM	2426	CG	TYR	B	100	3.236	15.589	-23.794	1.00	68.56	6	C
ATOM	2427	CD1	TYR	B	100	3.109	14.415	-23.065	1.00	67.74	6	C
ATOM	2428	CE1	TYR	B	100	2.868	14.441	-21.703	1.00	67.47	6	C
ATOM	2429	CZ	TYR	B	100	2.752	15.655	-21.054	1.00	67.56	6	C
ATOM	2430	OH	TYR	B	100	2.513	15.691	-19.699	1.00	67.02	8	O
ATOM	2431	CE2	TYR	B	100	2.879	16.834	-21.758	1.00	67.71	6	C
ATOM	2432	CD2	TYR	B	100	3.119	16.796	-23.119	1.00	68.03	6	C
ATOM	2433	C	TYR	B	100	1.083	15.713	-25.941	1.00	70.07	6	C
ATOM	2434	O	TYR	B	100	0.976	16.854	-26.392	1.00	70.07	8	O
ATOM	2435	N	ILE	B	101	0.099	15.093	-25.298	1.00	70.74	7	N
ATOM	2436	CA	ILE	B	101	-1.202	15.720	-25.093	1.00	71.51	6	C
ATOM	2437	CB	ILE	B	101	-2.311	14.944	-25.840	1.00	71.47	6	C
ATOM	2438	CG1	ILE	B	101	-1.852	14.522	-27.240	1.00	71.40	6	C
ATOM	2439	CD1	ILE	B	101	-1.252	13.132	-27.298	1.00	71.02	6	C
ATOM	2440	CG2	ILE	B	101	-3.583	15.769	-25.915	1.00	71.48	6	C
ATOM	2441	C	ILE	B	101	-1.551	15.793	-23.613	1.00	72.16	6	C
ATOM	2442	O	ILE	B	101	-1.736	14.764	-22.966	1.00	72.17	8	O

```
ATOM   2443  N   GLU B 102      -1.650  17.009 -23.083  1.00 73.07    7      N
ATOM   2444  CA  GLU B 102      -1.990  17.206 -21.674  1.00 73.99    6      C
ATOM   2445  CB  GLU B 102      -1.534  18.587 -21.189  1.00 73.96    6      C
ATOM   2446  CG  GLU B 102      -0.031  18.699 -20.984  1.00 74.39    6      C
ATOM   2447  CD  GLU B 102       0.396  20.050 -20.444  1.00 74.91    6      C
ATOM   2448  OE1 GLU B 102       0.630  20.157 -19.222  1.00 75.31    8      O
ATOM   2449  OE2 GLU B 102       0.506  21.005 -21.242  1.00 74.86    8      O
ATOM   2450  C   GLU B 102      -3.483  17.004 -21.403  1.00 74.52    6      C
ATOM   2451  O   GLU B 102      -4.286  16.922 -22.334  1.00 74.54    8      O
ATOM   2452  N   ARG B 103      -3.842  16.923 -20.123  1.00 75.28    7      N
ATOM   2453  CA  ARG B 103      -5.227  16.706 -19.704  1.00 76.03    6      C
ATOM   2454  CB  ARG B 103      -5.381  16.978 -18.206  1.00 76.14    6      C
ATOM   2455  CG  ARG B 103      -4.074  17.057 -17.434  1.00 76.75    6      C
ATOM   2456  CD  ARG B 103      -4.244  17.442 -15.969  1.00 77.80    6      C
ATOM   2457  NE  ARG B 103      -4.767  16.336 -15.170  1.00 78.42    7      N
ATOM   2458  CZ  ARG B 103      -5.251  16.462 -13.941  1.00 78.66    6      C
ATOM   2459  NH1 ARG B 103      -5.289  17.653 -13.357  1.00 78.73    7      N
ATOM   2460  NH2 ARG B 103      -5.699  15.395 -13.294  1.00 78.70    7      N
ATOM   2461  C   ARG B 103      -6.174  17.624 -20.460  1.00 76.38    6      C
ATOM   2462  O   ARG B 103      -7.267  17.222 -20.862  1.00 76.42    8      O
ATOM   2463  N   ASP B 104      -5.734  18.864 -20.646  1.00 76.80    7      N
ATOM   2464  CA  ASP B 104      -6.528  19.884 -21.315  1.00 77.26    6      C
ATOM   2465  CB  ASP B 104      -5.940  21.265 -21.024  1.00 77.33    6      C
ATOM   2466  CG  ASP B 104      -5.645  21.468 -19.548  1.00 77.74    6      C
ATOM   2467  OD1 ASP B 104      -6.428  20.963 -18.713  1.00 78.08    8      O
ATOM   2468  OD2 ASP B 104      -4.656  22.107 -19.126  1.00 78.02    8      O
ATOM   2469  C   ASP B 104      -6.639  19.654 -22.821  1.00 77.45    6      C
ATOM   2470  O   ASP B 104      -7.158  20.502 -23.548  1.00 77.53    8      O
ATOM   2471  N   GLY B 105      -6.149  18.506 -23.284  1.00 77.62    7      N
ATOM   2472  CA  GLY B 105      -6.222  18.146 -24.689  1.00 77.76    6      C
ATOM   2473  C   GLY B 105      -5.246  18.881 -25.589  1.00 77.96    6      C
ATOM   2474  O   GLY B 105      -5.221  18.656 -26.801  1.00 77.92    8      O
ATOM   2475  N   THR B 106      -4.438  19.758 -25.001  1.00 78.10    7      N
ATOM   2476  CA  THR B 106      -3.467  20.533 -25.766  1.00 78.26    6      C
ATOM   2477  CB  THR B 106      -2.848  21.640 -24.890  1.00 78.26    6      C
ATOM   2478  OG1 THR B 106      -3.860  22.589 -24.530  1.00 78.31    8      O
ATOM   2479  CG2 THR B 106      -1.861  22.471 -25.697  1.00 78.25    6      C
ATOM   2480  C   THR B 106      -2.371  19.645 -26.351  1.00 78.36    6      C
ATOM   2481  O   THR B 106      -1.687  18.921 -25.625  1.00 78.39    8      O
ATOM   2482  N   GLU B 107      -2.211  19.710 -27.669  1.00 78.42    7      N
ATOM   2483  CA  GLU B 107      -1.207  18.915 -28.365  1.00 78.51    6      C
ATOM   2484  CB  GLU B 107      -1.568  18.797 -29.847  1.00 78.54    6      C
ATOM   2485  CG  GLU B 107      -3.050  18.591 -30.124  1.00 78.80    6      C
ATOM   2486  CD  GLU B 107      -3.453  17.130 -30.142  1.00 79.26    6      C
ATOM   2487  OE1 GLU B 107      -2.556  16.267 -30.240  1.00 79.50    8      O
ATOM   2488  OE2 GLU B 107      -4.667  16.844 -30.065  1.00 79.53    8      O
ATOM   2489  C   GLU B 107       0.180  19.541 -28.226  1.00 78.51    6      C
ATOM   2490  O   GLU B 107       0.318  20.665 -27.748  1.00 78.62    8      O
ATOM   2491  N   SER B 108       1.199  18.800 -28.652  1.00 78.50    7      N
ATOM   2492  CA  SER B 108       2.585  19.260 -28.626  1.00 78.45    6      C
ATOM   2493  CB  SER B 108       3.051  19.543 -27.199  1.00 78.46    6      C
ATOM   2494  OG  SER B 108       3.040  18.365 -26.415  1.00 78.62    8      O
ATOM   2495  C   SER B 108       3.461  18.193 -29.270  1.00 78.43    6      C
ATOM   2496  O   SER B 108       3.515  17.057 -28.803  1.00 78.45    8      O
ATOM   2497  N   TYR B 109       4.152  18.564 -30.341  1.00 78.38    7      N
ATOM   2498  CA  TYR B 109       4.948  17.608 -31.101  1.00 78.34    6      C
ATOM   2499  CB  TYR B 109       4.589  17.716 -32.585  1.00 78.39    6      C
```

ATOM	2500	CG	TYR	B	109	3.099	17.874	-32.805	1.00	78.59	6	C
ATOM	2501	CD1	TYR	B	109	2.483	19.113	-32.660	1.00	78.75	6	C
ATOM	2502	CE1	TYR	B	109	1.121	19.262	-32.845	1.00	78.95	6	C
ATOM	2503	CZ	TYR	B	109	0.353	18.164	-33.175	1.00	79.00	6	C
ATOM	2504	OH	TYR	B	109	-1.003	18.310	-33.361	1.00	79.25	8	O
ATOM	2505	CE2	TYR	B	109	0.938	16.923	-33.319	1.00	78.96	6	C
ATOM	2506	CD2	TYR	B	109	2.303	16.782	-33.129	1.00	78.86	6	C
ATOM	2507	C	TYR	B	109	6.449	17.775	-30.881	1.00	78.23	6	C
ATOM	2508	O	TYR	B	109	7.040	18.780	-31.276	1.00	78.29	8	O
ATOM	2509	N	LEU	B	110	7.057	16.780	-30.243	1.00	78.03	7	N
ATOM	2510	CA	LEU	B	110	8.485	16.813	-29.947	1.00	77.83	6	C
ATOM	2511	CB	LEU	B	110	8.724	16.946	-28.439	1.00	77.87	6	C
ATOM	2512	CG	LEU	B	110	8.115	15.884	-27.517	1.00	77.92	6	C
ATOM	2513	CD1	LEU	B	110	8.880	15.816	-26.203	1.00	77.91	6	C
ATOM	2514	CD2	LEU	B	110	6.636	16.148	-27.269	1.00	77.94	6	C
ATOM	2515	C	LEU	B	110	9.209	15.583	-30.490	1.00	77.67	6	C
ATOM	2516	O	LEU	B	110	8.751	14.952	-31.443	1.00	77.63	8	O
ATOM	2517	N	THR	B	111	10.342	15.251	-29.877	1.00	77.46	7	N
ATOM	2518	CA	THR	B	111	11.153	14.119	-30.311	1.00	77.27	6	C
ATOM	2519	CB	THR	B	111	12.266	14.600	-31.267	1.00	77.30	6	C
ATOM	2520	OG1	THR	B	111	11.832	15.769	-31.973	1.00	77.54	8	O
ATOM	2521	CG2	THR	B	111	12.498	13.589	-32.373	1.00	77.32	6	C
ATOM	2522	C	THR	B	111	11.787	13.416	-29.115	1.00	77.06	6	C
ATOM	2523	O	THR	B	111	11.875	13.985	-28.027	1.00	77.07	8	O
ATOM	2524	N	VAL	B	112	12.218	12.174	-29.313	1.00	76.82	7	N
ATOM	2525	CA	VAL	B	112	12.906	11.438	-28.260	1.00	76.62	6	C
ATOM	2526	CB	VAL	B	112	12.893	9.915	-28.505	1.00	76.66	6	C
ATOM	2527	CG1	VAL	B	112	13.780	9.202	-27.498	1.00	76.61	6	C
ATOM	2528	CG2	VAL	B	112	11.473	9.370	-28.433	1.00	76.72	6	C
ATOM	2529	C	VAL	B	112	14.341	11.943	-28.224	1.00	76.45	6	C
ATOM	2530	O	VAL	B	112	15.005	11.911	-27.186	1.00	76.43	8	O
ATOM	2531	N	SER	B	113	14.806	12.419	-29.375	1.00	76.23	7	N
ATOM	2532	CA	SER	B	113	16.147	12.972	-29.501	1.00	76.03	6	C
ATOM	2533	CB	SER	B	113	16.628	12.901	-30.953	1.00	76.07	6	C
ATOM	2534	OG	SER	B	113	15.670	13.448	-31.844	1.00	76.02	8	O
ATOM	2535	C	SER	B	113	16.169	14.411	-28.996	1.00	75.80	6	C
ATOM	2536	O	SER	B	113	17.219	15.051	-28.953	1.00	75.73	8	O
ATOM	2537	N	SER	B	114	14.996	14.911	-28.616	1.00	75.52	7	N
ATOM	2538	CA	SER	B	114	14.871	16.254	-28.068	1.00	75.28	6	C
ATOM	2539	CB	SER	B	114	13.576	16.910	-28.540	1.00	75.35	6	C
ATOM	2540	OG	SER	B	114	12.455	16.348	-27.882	1.00	75.61	8	O
ATOM	2541	C	SER	B	114	14.879	16.160	-26.551	1.00	75.02	6	C
ATOM	2542	O	SER	B	114	14.202	16.931	-25.869	1.00	74.97	8	O
ATOM	2543	N	HIS	B	115	15.647	15.197	-26.046	1.00	74.69	7	N
ATOM	2544	CA	HIS	B	115	15.804	14.914	-24.617	1.00	74.31	6	C
ATOM	2545	CB	HIS	B	115	17.292	14.925	-24.250	1.00	74.43	6	C
ATOM	2546	CG	HIS	B	115	17.641	14.033	-23.099	1.00	74.97	6	C
ATOM	2547	ND1	HIS	B	115	18.750	13.214	-23.104	1.00	75.40	7	N
ATOM	2548	CE1	HIS	B	115	18.808	12.547	-21.965	1.00	75.64	6	C
ATOM	2549	NE2	HIS	B	115	17.776	12.904	-21.221	1.00	75.94	7	N
ATOM	2550	CD2	HIS	B	115	17.030	13.833	-21.907	1.00	75.53	6	C
ATOM	2551	C	HIS	B	115	15.029	15.834	-23.669	1.00	73.80	6	C
ATOM	2552	O	HIS	B	115	15.630	16.638	-22.954	1.00	73.83	8	O
ATOM	2553	N	PRO	B	116	13.703	15.710	-23.656	1.00	73.26	7	N
ATOM	2554	CA	PRO	B	116	12.855	16.537	-22.789	1.00	72.72	6	C
ATOM	2555	CB	PRO	B	116	11.443	16.154	-23.236	1.00	72.81	6	C
ATOM	2556	CG	PRO	B	116	11.605	14.775	-23.740	1.00	73.08	6	C

ATOM	2557	CD	PRO	B	116	12.900	14.797	-24.492	1.00	73.17	6	C
ATOM	2558	C	PRO	B	116	13.037	16.192	-21.317	1.00	72.09	6	C
ATOM	2559	O	PRO	B	116	12.667	15.096	-20.904	1.00	72.06	8	O
ATOM	2560	N	ASN	B	117	13.598	17.114	-20.540	1.00	71.24	7	N
ATOM	2561	CA	ASN	B	117	13.809	16.872	-19.119	1.00	70.39	6	C
ATOM	2562	CB	ASN	B	117	14.694	17.958	-18.509	1.00	70.52	6	C
ATOM	2563	CG	ASN	B	117	16.127	17.877	-18.994	1.00	70.84	6	C
ATOM	2564	OD1	ASN	B	117	16.923	17.091	-18.480	1.00	71.28	8	O
ATOM	2565	ND2	ASN	B	117	16.462	18.685	-19.994	1.00	70.97	7	N
ATOM	2566	C	ASN	B	117	12.489	16.767	-18.367	1.00	69.59	6	C
ATOM	2567	O	ASN	B	117	12.315	15.894	-17.516	1.00	69.52	8	O
ATOM	2568	N	ALA	B	118	11.560	17.660	-18.691	1.00	68.62	7	N
ATOM	2569	CA	ALA	B	118	10.242	17.645	-18.073	1.00	67.70	6	C
ATOM	2570	CB	ALA	B	118	9.438	18.858	-18.511	1.00	67.77	6	C
ATOM	2571	C	ALA	B	118	9.509	16.354	-18.434	1.00	66.98	6	C
ATOM	2572	O	ALA	B	118	8.786	15.786	-17.614	1.00	66.84	8	O
ATOM	2573	N	LEU	B	119	9.715	15.890	-19.664	1.00	66.05	7	N
ATOM	2574	CA	LEU	B	119	9.073	14.670	-20.148	1.00	65.14	6	C
ATOM	2575	CB	LEU	B	119	8.456	14.902	-21.531	1.00	65.24	6	C
ATOM	2576	CG	LEU	B	119	7.357	15.965	-21.601	1.00	65.57	6	C
ATOM	2577	CD1	LEU	B	119	6.827	16.114	-23.020	1.00	65.85	6	C
ATOM	2578	CD2	LEU	B	119	6.231	15.626	-20.636	1.00	65.80	6	C
ATOM	2579	C	LEU	B	119	10.033	13.486	-20.204	1.00	64.34	6	C
ATOM	2580	O	LEU	B	119	9.884	12.604	-21.046	1.00	64.28	8	O
ATOM	2581	N	MET	B	120	11.013	13.466	-19.305	1.00	63.32	7	N
ATOM	2582	CA	MET	B	120	11.990	12.378	-19.269	1.00	62.27	6	C
ATOM	2583	CB	MET	B	120	13.271	12.809	-18.551	1.00	62.47	6	C
ATOM	2584	CG	MET	B	120	14.453	13.018	-19.473	1.00	63.24	6	C
ATOM	2585	SD	MET	B	120	14.822	11.543	-20.454	1.00	65.13	16	S
ATOM	2586	CE	MET	B	120	15.182	10.353	-19.163	1.00	65.06	6	C
ATOM	2587	C	MET	B	120	11.445	11.118	-18.616	1.00	61.30	6	C
ATOM	2588	O	MET	B	120	11.685	10.011	-19.096	1.00	61.03	8	O
ATOM	2589	N	LYS	B	121	10.728	11.288	-17.512	1.00	60.15	7	N
ATOM	2590	CA	LYS	B	121	10.168	10.152	-16.795	1.00	59.20	6	C
ATOM	2591	CB	LYS	B	121	9.682	10.571	-15.403	1.00	59.38	6	C
ATOM	2592	CG	LYS	B	121	10.772	11.136	-14.489	1.00	59.63	6	C
ATOM	2593	CD	LYS	B	121	10.256	11.300	-13.062	1.00	60.08	6	C
ATOM	2594	CE	LYS	B	121	11.338	11.818	-12.119	1.00	60.74	6	C
ATOM	2595	NZ	LYS	B	121	11.682	13.251	-12.368	1.00	60.83	7	N
ATOM	2596	C	LYS	B	121	9.026	9.523	-17.588	1.00	58.39	6	C
ATOM	2597	O	LYS	B	121	8.823	8.310	-17.536	1.00	58.28	8	O
ATOM	2598	N	LYS	B	122	8.285	10.348	-18.324	1.00	57.44	7	N
ATOM	2599	CA	LYS	B	122	7.166	9.852	-19.125	1.00	56.58	6	C
ATOM	2600	CB	LYS	B	122	6.204	10.987	-19.499	1.00	56.59	6	C
ATOM	2601	CG	LYS	B	122	5.258	11.375	-18.362	1.00	56.35	6	C
ATOM	2602	CD	LYS	B	122	4.421	12.604	-18.684	1.00	56.34	6	C
ATOM	2603	CE	LYS	B	122	3.443	12.907	-17.547	1.00	56.68	6	C
ATOM	2604	NZ	LYS	B	122	2.607	14.115	-17.796	1.00	55.88	7	N
ATOM	2605	C	LYS	B	122	7.655	9.097	-20.363	1.00	56.03	6	C
ATOM	2606	O	LYS	B	122	7.179	8.000	-20.660	1.00	55.84	8	O
ATOM	2607	N	ILE	B	123	8.613	9.686	-21.074	1.00	55.27	7	N
ATOM	2608	CA	ILE	B	123	9.200	9.045	-22.247	1.00	54.71	6	C
ATOM	2609	CB	ILE	B	123	10.249	9.962	-22.902	1.00	54.74	6	C
ATOM	2610	CG1	ILE	B	123	9.563	11.042	-23.736	1.00	55.03	6	C
ATOM	2611	CD1	ILE	B	123	10.521	11.855	-24.568	1.00	55.34	6	C
ATOM	2612	CG2	ILE	B	123	11.199	9.156	-23.773	1.00	54.92	6	C
ATOM	2613	C	ILE	B	123	9.837	7.718	-21.863	1.00	54.03	6	C

ATOM	2614	O	ILE	B	123	9.676	6.712	-22.555	1.00	54.00	8	O
ATOM	2615	N	THR	B	124	10.560	7.721	-20.750	1.00	53.37	7	N
ATOM	2616	CA	THR	B	124	11.200	6.510	-20.255	1.00	52.73	6	C
ATOM	2617	CB	THR	B	124	12.011	6.810	-18.976	1.00	52.82	6	C
ATOM	2618	OG1	THR	B	124	13.171	7.577	-19.317	1.00	51.93	8	O
ATOM	2619	CG2	THR	B	124	12.587	5.531	-18.391	1.00	52.26	6	C
ATOM	2620	C	THR	B	124	10.154	5.439	-19.991	1.00	52.58	6	C
ATOM	2621	O	THR	B	124	10.320	4.285	-20.388	1.00	52.40	8	O
ATOM	2622	N	LEU	B	125	9.072	5.827	-19.324	1.00	52.52	7	N
ATOM	2623	CA	LEU	B	125	7.981	4.900	-19.051	1.00	52.53	6	C
ATOM	2624	CB	LEU	B	125	6.868	5.589	-18.264	1.00	52.34	6	C
ATOM	2625	CG	LEU	B	125	6.919	5.415	-16.746	1.00	52.83	6	C
ATOM	2626	CD1	LEU	B	125	5.986	6.401	-16.059	1.00	52.57	6	C
ATOM	2627	CD2	LEU	B	125	6.568	3.981	-16.361	1.00	52.79	6	C
ATOM	2628	C	LEU	B	125	7.433	4.328	-20.351	1.00	52.47	6	C
ATOM	2629	O	LEU	B	125	7.090	3.149	-20.422	1.00	52.35	8	O
ATOM	2630	N	LEU	B	126	7.369	5.164	-21.382	1.00	52.61	7	N
ATOM	2631	CA	LEU	B	126	6.852	4.735	-22.678	1.00	52.77	6	C
ATOM	2632	CB	LEU	B	126	6.673	5.927	-23.617	1.00	52.93	6	C
ATOM	2633	CG	LEU	B	126	5.952	5.609	-24.928	1.00	53.38	6	C
ATOM	2634	CD1	LEU	B	126	4.676	4.832	-24.644	1.00	54.11	6	C
ATOM	2635	CD2	LEU	B	126	5.642	6.875	-25.714	1.00	53.94	6	C
ATOM	2636	C	LEU	B	126	7.759	3.693	-23.326	1.00	52.83	6	C
ATOM	2637	O	LEU	B	126	7.284	2.795	-24.021	1.00	52.65	8	O
ATOM	2638	N	LYS	B	127	9.064	3.819	-23.095	1.00	52.84	7	N
ATOM	2639	CA	LYS	B	127	10.030	2.872	-23.640	1.00	52.93	6	C
ATOM	2640	CB	LYS	B	127	11.457	3.327	-23.346	1.00	53.06	6	C
ATOM	2641	CG	LYS	B	127	11.788	4.730	-23.807	1.00	53.98	6	C
ATOM	2642	CD	LYS	B	127	12.228	4.752	-25.255	1.00	55.23	6	C
ATOM	2643	CE	LYS	B	127	13.246	5.862	-25.484	1.00	55.58	6	C
ATOM	2644	NZ	LYS	B	127	13.877	5.784	-26.833	1.00	56.15	7	N
ATOM	2645	C	LYS	B	127	9.815	1.497	-23.024	1.00	52.76	6	C
ATOM	2646	O	LYS	B	127	9.772	0.489	-23.732	1.00	52.74	8	O
ATOM	2647	N	TYR	B	128	9.693	1.467	-21.701	1.00	52.52	7	N
ATOM	2648	CA	TYR	B	128	9.475	0.221	-20.977	1.00	52.44	6	C
ATOM	2649	CB	TYR	B	128	9.321	0.493	-19.478	1.00	52.59	6	C
ATOM	2650	CG	TYR	B	128	8.920	-0.707	-18.643	1.00	53.23	6	C
ATOM	2651	CD1	TYR	B	128	7.627	-0.829	-18.151	1.00	54.30	6	C
ATOM	2652	CE1	TYR	B	128	7.252	-1.915	-17.385	1.00	54.61	6	C
ATOM	2653	CZ	TYR	B	128	8.171	-2.894	-17.095	1.00	54.75	6	C
ATOM	2654	OH	TYR	B	128	7.788	-3.972	-16.329	1.00	55.15	8	O
ATOM	2655	CE2	TYR	B	128	9.466	-2.798	-17.563	1.00	54.54	6	C
ATOM	2656	CD2	TYR	B	128	9.834	-1.706	-18.332	1.00	54.10	6	C
ATOM	2657	C	TYR	B	128	8.258	-0.515	-21.530	1.00	52.27	6	C
ATOM	2658	O	TYR	B	128	8.344	-1.694	-21.867	1.00	52.20	8	O
ATOM	2659	N	PHE	B	129	7.132	0.188	-21.635	1.00	51.96	7	N
ATOM	2660	CA	PHE	B	129	5.907	-0.407	-22.162	1.00	51.77	6	C
ATOM	2661	CB	PHE	B	129	4.800	0.646	-22.261	1.00	51.70	6	C
ATOM	2662	CG	PHE	B	129	4.180	0.997	-20.942	1.00	51.57	6	C
ATOM	2663	CD1	PHE	B	129	3.995	2.320	-20.577	1.00	51.51	6	C
ATOM	2664	CE1	PHE	B	129	3.420	2.647	-19.358	1.00	51.58	6	C
ATOM	2665	CZ	PHE	B	129	3.024	1.648	-18.495	1.00	51.40	6	C
ATOM	2666	CE2	PHE	B	129	3.203	0.325	-18.850	1.00	51.91	6	C
ATOM	2667	CD2	PHE	B	129	3.778	0.003	-20.067	1.00	51.64	6	C
ATOM	2668	C	PHE	B	129	6.154	-1.040	-23.527	1.00	51.63	6	C
ATOM	2669	O	PHE	B	129	5.849	-2.210	-23.742	1.00	51.43	8	O
ATOM	2670	N	ARG	B	130	6.716	-0.251	-24.438	1.00	51.76	7	N

```
ATOM   2671  CA  ARG B 130       7.029  -0.712 -25.782  1.00 52.09   6       C
ATOM   2672  CB  ARG B 130       7.826   0.350 -26.540  1.00 52.12   6       C
ATOM   2673  CG  ARG B 130       8.353  -0.128 -27.886  1.00 52.68   6       C
ATOM   2674  CD  ARG B 130       9.224   0.881 -28.613  1.00 53.70   6       C
ATOM   2675  NE  ARG B 130      10.443   1.189 -27.868  1.00 54.71   7       N
ATOM   2676  CZ  ARG B 130      11.375   2.032 -28.287  1.00 55.07   6       C
ATOM   2677  NH1 ARG B 130      11.230   2.654 -29.451  1.00 55.48   7       N
ATOM   2678  NH2 ARG B 130      12.454   2.256 -27.547  1.00 55.58   7       N
ATOM   2679  C   ARG B 130       7.811  -2.015 -25.750  1.00 52.16   6       C
ATOM   2680  O   ARG B 130       7.408  -3.007 -26.359  1.00 52.02   8       O
ATOM   2681  N   ASN B 131       8.933  -2.005 -25.035  1.00 52.22   7       N
ATOM   2682  CA  ASN B 131       9.780  -3.184 -24.921  1.00 52.32   6       C
ATOM   2683  CB  ASN B 131      11.061  -2.854 -24.144  1.00 52.50   6       C
ATOM   2684  CG  ASN B 131      11.847  -1.714 -24.768  1.00 53.32   6       C
ATOM   2685  OD1 ASN B 131      11.504  -1.220 -25.845  1.00 54.22   8       O
ATOM   2686  ND2 ASN B 131      12.910  -1.291 -24.092  1.00 54.67   7       N
ATOM   2687  C   ASN B 131       9.054  -4.352 -24.260  1.00 52.15   6       C
ATOM   2688  O   ASN B 131       9.245  -5.507 -24.644  1.00 52.01   8       O
ATOM   2689  N   TYR B 132       8.222  -4.052 -23.266  1.00 51.97   7       N
ATOM   2690  CA  TYR B 132       7.481  -5.100 -22.571  1.00 52.06   6       C
ATOM   2691  CB  TYR B 132       6.742  -4.542 -21.354  1.00 52.17   6       C
ATOM   2692  CG  TYR B 132       5.904  -5.575 -20.632  1.00 52.63   6       C
ATOM   2693  CD1 TYR B 132       6.388  -6.228 -19.510  1.00 52.75   6       C
ATOM   2694  CE1 TYR B 132       5.626  -7.173 -18.849  1.00 53.37   6       C
ATOM   2695  CZ  TYR B 132       4.363  -7.479 -19.313  1.00 53.58   6       C
ATOM   2696  OH  TYR B 132       3.603  -8.421 -18.658  1.00 54.12   8       O
ATOM   2697  CE2 TYR B 132       3.859  -6.847 -20.427  1.00 53.55   6       C
ATOM   2698  CD2 TYR B 132       4.628  -5.901 -21.080  1.00 53.51   6       C
ATOM   2699  C   TYR B 132       6.490  -5.799 -23.499  1.00 51.98   6       C
ATOM   2700  O   TYR B 132       6.414  -7.023 -23.527  1.00 51.86   8       O
ATOM   2701  N   MET B 133       5.727  -5.015 -24.252  1.00 52.02   7       N
ATOM   2702  CA  MET B 133       4.738  -5.578 -25.167  1.00 52.00   6       C
ATOM   2703  CB  MET B 133       3.871  -4.468 -25.768  1.00 51.99   6       C
ATOM   2704  CG  MET B 133       3.024  -3.728 -24.737  1.00 51.75   6       C
ATOM   2705  SD  MET B 133       2.056  -2.335 -25.406  1.00 50.86  16       S
ATOM   2706  CE  MET B 133       3.353  -1.216 -25.922  1.00 51.88   6       C
ATOM   2707  C   MET B 133       5.424  -6.395 -26.261  1.00 52.18   6       C
ATOM   2708  O   MET B 133       5.016  -7.517 -26.563  1.00 52.10   8       O
ATOM   2709  N   SER B 134       6.486  -5.829 -26.829  1.00 52.40   7       N
ATOM   2710  CA  SER B 134       7.260  -6.479 -27.880  1.00 52.62   6       C
ATOM   2711  CB  SER B 134       8.332  -5.523 -28.410  1.00 52.61   6       C
ATOM   2712  OG  SER B 134       9.154  -6.146 -29.382  1.00 53.06   8       O
ATOM   2713  C   SER B 134       7.904  -7.782 -27.408  1.00 52.68   6       C
ATOM   2714  O   SER B 134       8.330  -8.601 -28.223  1.00 52.83   8       O
ATOM   2715  N   GLU B 135       7.953  -7.977 -26.095  1.00 52.77   7       N
ATOM   2716  CA  GLU B 135       8.563  -9.171 -25.517  1.00 52.92   6       C
ATOM   2717  CB  GLU B 135       9.353  -8.814 -24.256  1.00 53.26   6       C
ATOM   2718  CG  GLU B 135      10.839  -8.604 -24.488  1.00 54.61   6       C
ATOM   2719  CD  GLU B 135      11.646  -8.804 -23.224  1.00 56.43   6       C
ATOM   2720  OE1 GLU B 135      11.356  -8.121 -22.219  1.00 57.82   8       O
ATOM   2721  OE2 GLU B 135      12.563  -9.650 -23.234  1.00 57.68   8       O
ATOM   2722  C   GLU B 135       7.597 -10.309 -25.187  1.00 52.64   6       C
ATOM   2723  O   GLU B 135       7.868 -11.463 -25.518  1.00 52.69   8       O
ATOM   2724  N   HIS B 136       6.480  -9.988 -24.539  1.00 52.18   7       N
ATOM   2725  CA  HIS B 136       5.550 -11.015 -24.066  1.00 51.81   6       C
ATOM   2726  CB  HIS B 136       5.302 -10.838 -22.564  1.00 52.15   6       C
ATOM   2727  CG  HIS B 136       6.531 -10.962 -21.717  1.00 52.92   6       C
```

ATOM	2728	ND1	HIS	B	136	6.978	-12.170	-21.230	1.00	53.81	7	N
ATOM	2729	CE1	HIS	B	136	8.069	-11.975	-20.509	1.00	54.28	6	C
ATOM	2730	NE2	HIS	B	136	8.340	-10.681	-20.505	1.00	54.06	7	N
ATOM	2731	CD2	HIS	B	136	7.391	-10.025	-21.252	1.00	53.73	6	C
ATOM	2732	C	HIS	B	136	4.178	-11.053	-24.744	1.00	51.31	6	C
ATOM	2733	O	HIS	B	136	3.456	-12.044	-24.620	1.00	51.17	8	O
ATOM	2734	N	LEU	B	137	3.812	-9.988	-25.447	1.00	50.72	7	N
ATOM	2735	CA	LEU	B	137	2.442	-9.878	-25.960	1.00	50.21	6	C
ATOM	2736	CB	LEU	B	137	1.858	-8.517	-25.569	1.00	49.98	6	C
ATOM	2737	CG	LEU	B	137	1.925	-8.177	-24.078	1.00	49.21	6	C
ATOM	2738	CD1	LEU	B	137	1.125	-6.918	-23.785	1.00	48.39	6	C
ATOM	2739	CD2	LEU	B	137	1.421	-9.335	-23.224	1.00	48.56	6	C
ATOM	2740	C	LEU	B	137	2.217	-10.138	-27.451	1.00	49.97	6	C
ATOM	2741	O	LEU	B	137	3.000	-9.713	-28.298	1.00	49.87	8	O
ATOM	**2742**	**N**	**LEU**	**B**	**138**	**1.111**	**-10.818**	**-27.749**	**1.00**	**49.94**	**7**	**N**
ATOM	**2743**	**CA**	**LEU**	**B**	**138**	**0.698**	**-11.120**	**-29.120**	**1.00**	**49.71**	**6**	**C**
ATOM	**2744**	**CB**	**LEU**	**B**	**138**	**-0.210**	**-12.351**	**-29.129**	**1.00**	**49.67**	**6**	**C**
ATOM	**2745**	**CG**	**LEU**	**B**	**138**	**-0.887**	**-12.698**	**-30.458**	**1.00**	**49.70**	**6**	**C**
ATOM	**2746**	**CD1**	**LEU**	**B**	**138**	**0.106**	**-13.338**	**-31.416**	**1.00**	**49.27**	**6**	**C**
ATOM	**2747**	**CD2**	**LEU**	**B**	**138**	**-2.073**	**-13.618**	**-30.222**	**1.00**	**49.98**	**6**	**C**
ATOM	**2748**	**C**	**LEU**	**B**	**138**	**-0.035**	**-9.942**	**-29.776**	**1.00**	**49.63**	**6**	**C**
ATOM	**2749**	**O**	**LEU**	**B**	**138**	**-0.911**	**-9.329**	**-29.172**	**1.00**	**49.34**	**8**	**O**
ATOM	2750	N	LYS	B	139	0.329	-9.645	-31.018	1.00	49.67	7	N
ATOM	2751	CA	LYS	B	139	-0.260	-8.546	-31.775	1.00	50.01	6	C
ATOM	2752	CB	LYS	B	139	0.691	-8.140	-32.904	1.00	49.94	6	C
ATOM	2753	CG	LYS	B	139	0.324	-6.867	-33.656	1.00	50.31	6	C
ATOM	2754	CD	LYS	B	139	1.516	-6.389	-34.478	1.00	50.49	6	C
ATOM	2755	CE	LYS	B	139	1.096	-5.589	-35.696	1.00	50.83	6	C
ATOM	2756	NZ	LYS	B	139	0.523	-4.273	-35.345	1.00	51.00	7	N
ATOM	2757	C	LYS	B	139	-1.634	-8.921	-32.342	1.00	50.29	6	C
ATOM	2758	O	LYS	B	139	-1.737	-9.750	-33.252	1.00	50.15	8	O
ATOM	2759	N	ALA	B	140	-2.682	-8.306	-31.796	1.00	50.50	7	N
ATOM	2760	CA	ALA	B	140	-4.054	-8.562	-32.234	1.00	50.83	6	C
ATOM	2761	CB	ALA	B	140	-5.050	-7.982	-31.239	1.00	50.79	6	C
ATOM	2762	C	ALA	B	140	-4.322	-8.010	-33.626	1.00	51.19	6	C
ATOM	2763	O	ALA	B	140	-3.855	-6.930	-33.979	1.00	51.15	8	O
ATOM	2764	N	GLY	B	141	-5.086	-8.759	-34.414	1.00	51.87	7	N
ATOM	2765	CA	GLY	B	141	-5.411	-8.348	-35.765	1.00	52.43	6	C
ATOM	2766	C	GLY	B	141	-4.195	-8.378	-36.665	1.00	53.11	6	C
ATOM	2767	O	GLY	B	141	-4.118	-7.639	-37.644	1.00	52.86	8	O
ATOM	2768	N	ALA	B	142	-3.239	-9.236	-36.329	1.00	53.86	7	N
ATOM	2769	CA	ALA	B	142	-2.027	-9.374	-37.125	1.00	54.88	6	C
ATOM	2770	CB	ALA	B	142	-1.108	-10.422	-36.516	1.00	54.80	6	C
ATOM	2771	C	ALA	B	142	-2.354	-9.729	-38.575	1.00	55.58	6	C
ATOM	2772	O	ALA	B	142	-1.614	-9.369	-39.490	1.00	55.55	8	O
ATOM	2773	N	ASN	B	143	-3.472	-10.424	-38.774	1.00	56.60	7	N
ATOM	2774	CA	ASN	B	143	-3.899	-10.838	-40.109	1.00	57.64	6	C
ATOM	2775	CB	ASN	B	143	-4.584	-12.208	-40.057	1.00	57.56	6	C
ATOM	2776	CG	ASN	B	143	-5.751	-12.246	-39.084	1.00	57.75	6	C
ATOM	2777	OD1	ASN	B	143	-5.854	-11.411	-38.182	1.00	57.67	8	O
ATOM	2778	ND2	ASN	B	143	-6.634	-13.224	-39.259	1.00	57.49	7	N
ATOM	2779	C	ASN	B	143	-4.808	-9.829	-40.806	1.00	58.43	6	C
ATOM	2780	O	ASN	B	143	-5.391	-10.130	-41.850	1.00	58.60	8	O
ATOM	2781	N	ILE	B	144	-4.927	-8.636	-40.231	1.00	59.33	7	N
ATOM	2782	CA	ILE	B	144	-5.757	-7.587	-40.814	1.00	60.27	6	C
ATOM	2783	CB	ILE	B	144	-6.665	-6.953	-39.735	1.00	60.15	6	C
ATOM	2784	CG1	ILE	B	144	-7.501	-8.025	-39.033	1.00	60.27	6	C

ATOM	2785	CD1	ILE	B	144	-8.419	-7.489	-37.949	1.00	59.65	6	C
ATOM	2786	CG2	ILE	B	144	-7.560	-5.887	-40.344	1.00	60.18	6	C
ATOM	2787	C	ILE	B	144	-4.896	-6.510	-41.466	1.00	61.06	6	C
ATOM	2788	O	ILE	B	144	-3.826	-6.175	-40.960	1.00	61.18	8	O
ATOM	2789	N	THR	B	145	-5.362	-5.971	-42.589	1.00	62.15	7	N
ATOM	2790	CA	THR	B	145	-4.656	-4.894	-43.280	1.00	63.24	6	C
ATOM	2791	CB	THR	B	145	-4.454	-5.233	-44.775	1.00	63.29	6	C
ATOM	2792	OG1	THR	B	145	-3.538	-6.327	-44.907	1.00	63.28	8	O
ATOM	2793	CG2	THR	B	145	-3.741	-4.092	-45.489	1.00	63.12	6	C
ATOM	2794	C	THR	B	145	-5.422	-3.578	-43.138	1.00	64.10	6	C
ATOM	2795	O	THR	B	145	-6.539	-3.447	-43.641	1.00	63.93	8	O
ATOM	2796	N	PRO	B	146	-4.812	-2.614	-42.449	1.00	65.06	7	N
ATOM	2797	CA	PRO	B	146	-5.414	-1.293	-42.203	1.00	65.86	6	C
ATOM	2798	CB	PRO	B	146	-4.308	-0.542	-41.457	1.00	65.86	6	C
ATOM	2799	CG	PRO	B	146	-3.478	-1.613	-40.847	1.00	65.50	6	C
ATOM	2800	CD	PRO	B	146	-3.484	-2.743	-41.826	1.00	64.99	6	C
ATOM	2801	C	PRO	B	146	-5.832	-0.513	-43.457	1.00	66.81	6	C
ATOM	2802	O	PRO	B	146	-5.676	-0.994	-44.581	1.00	66.84	8	O
ATOM	2803	N	ARG	B	147	-6.324	0.707	-43.244	1.00	67.90	7	N
ATOM	2804	CA	ARG	B	147	-6.918	1.522	-44.303	1.00	68.90	6	C
ATOM	2805	CB	ARG	B	147	-8.351	1.872	-43.891	1.00	68.76	6	C
ATOM	2806	CG	ARG	B	147	-9.312	2.113	-45.034	1.00	68.48	6	C
ATOM	2807	CD	ARG	B	147	-10.729	2.421	-44.577	1.00	68.19	6	C
ATOM	2808	NE	ARG	B	147	-10.853	3.743	-43.970	1.00	67.59	7	N
ATOM	2809	CZ	ARG	B	147	-11.582	4.006	-42.891	1.00	67.44	6	C
ATOM	2810	NH1	ARG	B	147	-12.251	3.036	-42.283	1.00	67.26	7	N
ATOM	2811	NH2	ARG	B	147	-11.642	5.240	-42.413	1.00	67.45	7	N
ATOM	2812	C	ARG	B	147	-6.155	2.809	-44.642	1.00	69.68	6	C
ATOM	2813	O	ARG	B	147	-4.973	2.944	-44.333	1.00	69.95	8	O
ATOM	2814	N	GLU	B	148	-6.854	3.750	-45.280	1.00	70.69	7	N
ATOM	2815	CA	GLU	B	148	-6.277	5.031	-45.699	1.00	71.50	6	C
ATOM	2816	CB	GLU	B	148	-6.978	5.552	-46.957	1.00	71.67	6	C
ATOM	2817	CG	GLU	B	148	-7.163	4.544	-48.078	1.00	72.40	6	C
ATOM	2818	CD	GLU	B	148	-8.089	5.069	-49.160	1.00	73.44	6	C
ATOM	2819	OE1	GLU	B	148	-8.789	6.073	-48.902	1.00	73.72	8	O
ATOM	2820	OE2	GLU	B	148	-8.116	4.482	-50.265	1.00	73.75	8	O
ATOM	2821	C	GLU	B	148	-6.388	6.110	-44.624	1.00	71.87	6	C
ATOM	2822	O	GLU	B	148	-7.396	6.199	-43.923	1.00	72.05	8	O
ATOM	2823	N	GLY	B	149	-5.359	6.947	-44.523	1.00	72.24	7	N
ATOM	2824	CA	GLY	B	149	-5.339	8.038	-43.564	1.00	72.61	6	C
ATOM	2825	C	GLY	B	149	-4.753	9.306	-44.162	1.00	72.89	6	C
ATOM	2826	O	GLY	B	149	-4.102	9.263	-45.209	1.00	72.91	8	O
ATOM	2827	N	ASP	B	150	-4.985	10.438	-43.500	1.00	73.09	7	N
ATOM	2828	CA	ASP	B	150	-4.475	11.726	-43.968	1.00	73.25	6	C
ATOM	2829	CB	ASP	B	150	-5.458	12.850	-43.637	1.00	73.46	6	C
ATOM	2830	CG	ASP	B	150	-6.855	12.569	-44.151	1.00	74.14	6	C
ATOM	2831	OD1	ASP	B	150	-6.979	11.880	-45.187	1.00	74.66	8	O
ATOM	2832	OD2	ASP	B	150	-7.886	12.992	-43.584	1.00	75.11	8	O
ATOM	2833	C	ASP	B	150	-3.102	12.022	-43.370	1.00	73.12	6	C
ATOM	2834	O	ASP	B	150	-2.960	12.868	-42.484	1.00	73.15	8	O
ATOM	2835	N	GLU	B	151	-2.099	11.315	-43.882	1.00	72.84	7	N
ATOM	2836	CA	GLU	B	151	-0.708	11.406	-43.435	1.00	72.56	6	C
ATOM	2837	CB	GLU	B	151	0.229	11.118	-44.613	1.00	72.70	6	C
ATOM	2838	CG	GLU	B	151	-0.106	9.841	-45.369	1.00	73.28	6	C
ATOM	2839	CD	GLU	B	151	0.772	9.637	-46.589	1.00	74.10	6	C
ATOM	2840	OE1	GLU	B	151	1.606	10.521	-46.876	1.00	74.33	8	O
ATOM	2841	OE2	GLU	B	151	0.627	8.593	-47.262	1.00	74.53	8	O

ATOM	2842	C	GLU	B	151	-0.274	12.704	-42.744	1.00	72.11	6	C
ATOM	2843	O	GLU	B	151	0.538	12.670	-41.817	1.00	72.16	8	O
ATOM	2844	N	LEU	B	152	-0.805	13.840	-43.189	1.00	71.49	7	N
ATOM	2845	CA	LEU	B	152	-0.397	15.141	-42.647	1.00	70.86	6	C
ATOM	2846	CB	LEU	B	152	-0.871	16.279	-43.557	1.00	71.02	6	C
ATOM	2847	CG	LEU	B	152	-0.300	16.285	-44.977	1.00	71.31	6	C
ATOM	2848	CD1	LEU	B	152	-0.840	17.469	-45.771	1.00	71.66	6	C
ATOM	2849	CD2	LEU	B	152	1.222	16.305	-44.946	1.00	71.67	6	C
ATOM	2850	C	LEU	B	152	-0.829	15.410	-41.201	1.00	70.20	6	C
ATOM	2851	O	LEU	B	152	-0.299	16.313	-40.551	1.00	70.25	8	O
ATOM	2852	N	ALA	B	153	-1.784	14.630	-40.703	1.00	69.21	7	N
ATOM	2853	CA	ALA	B	153	-2.261	14.796	-39.334	1.00	68.16	6	C
ATOM	2854	CB	ALA	B	153	-2.827	16.197	-39.132	1.00	68.31	6	C
ATOM	2855	C	ALA	B	153	-3.307	13.746	-38.978	1.00	67.34	6	C
ATOM	2856	O	ALA	B	153	-4.502	14.042	-38.936	1.00	67.43	8	O
ATOM	2857	N	ARG	B	154	-2.851	12.524	-38.720	1.00	66.04	7	N
ATOM	2858	CA	ARG	B	154	-3.748	11.428	-38.365	1.00	64.75	6	C
ATOM	2859	CB	ARG	B	154	-4.341	10.776	-39.617	1.00	64.95	6	C
ATOM	2860	CG	ARG	B	154	-5.369	11.623	-40.344	1.00	65.66	6	C
ATOM	2861	CD	ARG	B	154	-6.610	11.944	-39.533	1.00	66.89	6	C
ATOM	2862	NE	ARG	B	154	-7.517	12.814	-40.275	1.00	67.71	7	N
ATOM	2863	CZ	ARG	B	154	-8.735	13.142	-39.866	1.00	68.02	6	C
ATOM	2864	NH1	ARG	B	154	-9.197	12.675	-38.715	1.00	68.34	7	N
ATOM	2865	NH2	ARG	B	154	-9.493	13.940	-40.607	1.00	68.13	7	N
ATOM	2866	C	ARG	B	154	-3.048	10.365	-37.528	1.00	63.44	6	C
ATOM	2867	O	ARG	B	154	-2.103	9.717	-37.983	1.00	63.52	8	O
ATOM	2868	N	LEU	B	155	-3.532	10.194	-36.305	1.00	61.63	7	N
ATOM	2869	CA	LEU	B	155	-3.025	9.193	-35.375	1.00	59.76	6	C
ATOM	2870	CB	LEU	B	155	-1.506	9.246	-35.247	1.00	59.94	6	C
ATOM	2871	CG	LEU	B	155	-0.903	7.990	-34.617	1.00	60.13	6	C
ATOM	2872	CD1	LEU	B	155	-0.654	6.935	-35.679	1.00	60.60	6	C
ATOM	2873	CD2	LEU	B	155	0.385	8.318	-33.897	1.00	60.79	6	C
ATOM	2874	C	LEU	B	155	-3.670	9.501	-34.041	1.00	58.15	6	C
ATOM	2875	O	LEU	B	155	-3.347	10.505	-33.406	1.00	58.10	8	O
ATOM	2876	N	PRO	B	156	-4.587	8.635	-33.625	1.00	56.42	7	N
ATOM	2877	CA	PRO	B	156	-5.373	8.850	-32.413	1.00	54.95	6	C
ATOM	2878	CB	PRO	B	156	-6.534	7.884	-32.621	1.00	55.01	6	C
ATOM	2879	CG	PRO	B	156	-5.843	6.718	-33.220	1.00	55.56	6	C
ATOM	2880	CD	PRO	B	156	-4.956	7.366	-34.279	1.00	56.24	6	C
ATOM	2881	C	PRO	B	156	-4.633	8.472	-31.145	1.00	53.41	6	C
ATOM	2882	O	PRO	B	156	-3.687	7.689	-31.174	1.00	53.43	8	O
ATOM	2883	N	TYR	B	157	-5.075	9.041	-30.034	1.00	51.51	7	N
ATOM	2884	CA	TYR	B	157	-4.530	8.697	-28.735	1.00	49.68	6	C
ATOM	2885	CB	TYR	B	157	-3.965	9.931	-28.031	1.00	49.53	6	C
ATOM	2886	CG	TYR	B	157	-4.937	11.084	-27.898	1.00	48.93	6	C
ATOM	2887	CD1	TYR	B	157	-5.843	11.139	-26.849	1.00	48.66	6	C
ATOM	2888	CE1	TYR	B	157	-6.727	12.196	-26.721	1.00	47.81	6	C
ATOM	2889	CZ	TYR	B	157	-6.708	13.217	-27.646	1.00	47.98	6	C
ATOM	2890	OH	TYR	B	157	-7.583	14.276	-27.522	1.00	47.77	8	O
ATOM	2891	CE2	TYR	B	157	-5.814	13.188	-28.693	1.00	48.01	6	C
ATOM	2892	CD2	TYR	B	157	-4.934	12.128	-28.813	1.00	48.72	6	C
ATOM	2893	C	TYR	B	157	-5.664	8.102	-27.926	1.00	48.53	6	C
ATOM	2894	O	TYR	B	157	-6.829	8.241	-28.294	1.00	48.31	8	O
ATOM	2895	N	LEU	B	158	-5.332	7.439	-26.827	1.00	47.21	7	N
ATOM	2896	CA	LEU	B	158	-6.353	6.853	-25.975	1.00	46.08	6	C
ATOM	2897	CB	LEU	B	158	-5.738	5.803	-25.068	1.00	46.07	6	C
ATOM	2898	CG	LEU	B	158	-6.731	5.066	-24.180	1.00	45.30	6	C

```
ATOM   2899  CD1 LEU B 158      -7.716   4.278 -25.042  1.00 45.13      6      C
ATOM   2900  CD2 LEU B 158      -5.978   4.150 -23.247  1.00 44.78      6      C
ATOM   2901  C   LEU B 158      -7.028   7.918 -25.122  1.00 45.64      6      C
ATOM   2902  O   LEU B 158      -6.416   8.460 -24.201  1.00 45.58      8      O
ATOM   2903  N   ARG B 159      -8.288   8.217 -25.422  1.00 44.66      7      N
ATOM   2904  CA  ARG B 159      -9.013   9.220 -24.650  1.00 44.17      6      C
ATOM   2905  CB  ARG B 159     -10.315   9.610 -25.341  1.00 44.51      6      C
ATOM   2906  CG  ARG B 159     -10.975  10.832 -24.730  1.00 47.41      6      C
ATOM   2907  CD  ARG B 159     -12.466  10.915 -24.992  1.00 51.06      6      C
ATOM   2908  NE  ARG B 159     -12.853  12.232 -25.485  1.00 53.52      7      N
ATOM   2909  CZ  ARG B 159     -12.653  12.641 -26.733  1.00 55.08      6      C
ATOM   2910  NH1 ARG B 159     -12.065  11.835 -27.613  1.00 55.53      7      N
ATOM   2911  NH2 ARG B 159     -13.039  13.855 -27.105  1.00 55.21      7      N
ATOM   2912  C   ARG B 159      -9.315   8.688 -23.259  1.00 43.00      6      C
ATOM   2913  O   ARG B 159      -9.125   9.375 -22.257  1.00 42.82      8      O
ATOM   2914  N   THR B 160      -9.799   7.457 -23.203  1.00 41.62      7      N
ATOM   2915  CA  THR B 160     -10.102   6.827 -21.929  1.00 40.46      6      C
ATOM   2916  CB  THR B 160     -11.342   7.472 -21.271  1.00 40.57      6      C
ATOM   2917  OG1 THR B 160     -11.439   7.048 -19.902  1.00 40.80      8      O
ATOM   2918  CG2 THR B 160     -12.633   6.962 -21.911  1.00 41.26      6      C
ATOM   2919  C   THR B 160     -10.288   5.327 -22.108  1.00 39.68      6      C
ATOM   2920  O   THR B 160     -10.299   4.818 -23.231  1.00 38.81      8      O
ATOM   2921  N   TRP B 161     -10.424   4.625 -20.993  1.00 38.77      7      N
ATOM   2922  CA  TRP B 161     -10.577   3.185 -21.019  1.00 38.55      6      C
ATOM   2923  CB  TRP B 161      -9.227   2.508 -21.276  1.00 38.41      6      C
ATOM   2924  CG  TRP B 161      -8.281   2.658 -20.114  1.00 39.64      6      C
ATOM   2925  CD1 TRP B 161      -7.445   3.708 -19.870  1.00 40.03      6      C
ATOM   2926  NE1 TRP B 161      -6.749   3.499 -18.702  1.00 41.04      7      N
ATOM   2927  CE2 TRP B 161      -7.135   2.300 -18.163  1.00 40.87      6      C
ATOM   2928  CD2 TRP B 161      -8.105   1.745 -19.024  1.00 39.93      6      C
ATOM   2929  CE3 TRP B 161      -8.657   0.503 -18.694  1.00 40.64      6      C
ATOM   2930  CZ3 TRP B 161      -8.240  -0.130 -17.537  1.00 41.98      6      C
ATOM   2931  CH2 TRP B 161      -7.276   0.450 -16.702  1.00 41.80      6      C
ATOM   2932  CZ2 TRP B 161      -6.712   1.661 -16.999  1.00 41.26      6      C
ATOM   2933  C   TRP B 161     -11.096   2.741 -19.676  1.00 37.76      6      C
ATOM   2934  O   TRP B 161     -11.030   3.491 -18.706  1.00 37.63      8      O
ATOM   2935  N   PHE B 162     -11.637   1.531 -19.625  1.00 37.15      7      N
ATOM   2936  CA  PHE B 162     -12.058   0.936 -18.359  1.00 36.85      6      C
ATOM   2937  CB  PHE B 162     -13.321   1.594 -17.770  1.00 37.15      6      C
ATOM   2938  CG  PHE B 162     -14.592   1.319 -18.540  1.00 36.74      6      C
ATOM   2939  CD1 PHE B 162     -15.378   0.213 -18.246  1.00 36.22      6      C
ATOM   2940  CE1 PHE B 162     -16.549  -0.033 -18.944  1.00 37.13      6      C
ATOM   2941  CZ  PHE B 162     -16.956   0.835 -19.935  1.00 35.85      6      C
ATOM   2942  CE2 PHE B 162     -16.191   1.945 -20.235  1.00 36.28      6      C
ATOM   2943  CD2 PHE B 162     -15.011   2.185 -19.533  1.00 36.01      6      C
ATOM   2944  C   PHE B 162     -12.215  -0.566 -18.510  1.00 36.88      6      C
ATOM   2945  O   PHE B 162     -12.373  -1.070 -19.617  1.00 36.24      8      O
ATOM   2946  N   ARG B 163     -12.133  -1.285 -17.398  1.00 36.54      7      N
ATOM   2947  CA  ARG B 163     -12.281  -2.732 -17.446  1.00 36.80      6      C
ATOM   2948  CB  ARG B 163     -11.065  -3.427 -16.822  1.00 36.93      6      C
ATOM   2949  CG  ARG B 163     -10.863  -3.121 -15.340  1.00 37.83      6      C
ATOM   2950  CD  ARG B 163      -9.774  -3.974 -14.676  1.00 40.09      6      C
ATOM   2951  NE  ARG B 163      -8.523  -3.931 -15.431  1.00 40.80      7      N
ATOM   2952  CZ  ARG B 163      -7.968  -4.978 -16.032  1.00 41.56      6      C
ATOM   2953  NH1 ARG B 163      -8.543  -6.173 -15.976  1.00 41.79      7      N
ATOM   2954  NH2 ARG B 163      -6.826  -4.831 -16.689  1.00 41.68      7      N
ATOM   2955  C   ARG B 163     -13.541  -3.151 -16.714  1.00 36.35      6      C
```

```
ATOM   2956  O   ARG B 163     -14.005  -2.451 -15.820  1.00 36.32   8        O
ATOM   2957  N   THR B 164     -14.121  -4.268 -17.141  1.00 36.11   7        N
ATOM   2958  CA  THR B 164     -15.242  -4.881 -16.440  1.00 36.21   6        C
ATOM   2959  CB  THR B 164     -16.510  -4.900 -17.302  1.00 36.10   6        C
ATOM   2960  OG1 THR B 164     -16.329  -5.800 -18.409  1.00 36.37   8        O
ATOM   2961  CG2 THR B 164     -16.727  -3.544 -17.965  1.00 36.23   6        C
ATOM   2962  C   THR B 164     -14.785  -6.311 -16.163  1.00 36.56   6        C
ATOM   2963  O   THR B 164     -13.656  -6.665 -16.492  1.00 36.28   8        O
ATOM   2964  N   ARG B 165     -15.651  -7.130 -15.582  1.00 36.60   7        N
ATOM   2965  CA  ARG B 165     -15.292  -8.518 -15.306  1.00 37.60   6        C
ATOM   2966  CB  ARG B 165     -16.314  -9.166 -14.370  1.00 37.85   6        C
ATOM   2967  CG  ARG B 165     -16.517  -8.457 -13.039  1.00 38.71   6        C
ATOM   2968  CD  ARG B 165     -17.309  -9.283 -12.047  1.00 40.03   6        C
ATOM   2969  NE  ARG B 165     -16.648 -10.561 -11.799  1.00 42.08   7        N
ATOM   2970  CZ  ARG B 165     -17.224 -11.605 -11.210  1.00 43.13   6        C
ATOM   2971  NH1 ARG B 165     -18.485 -11.529 -10.799  1.00 43.55   7        N
ATOM   2972  NH2 ARG B 165     -16.538 -12.725 -11.028  1.00 42.32   7        N
ATOM   2973  C   ARG B 165     -15.202  -9.372 -16.564  1.00 37.61   6        C
ATOM   2974  O   ARG B 165     -14.737 -10.510 -16.502  1.00 38.14   8        O
ATOM   2975  N   SER B 166     -15.666  -8.843 -17.696  1.00 37.20   7        N
ATOM   2976  CA  SER B 166     -15.697  -9.618 -18.936  1.00 36.59   6        C
ATOM   2977  CB  SER B 166     -17.138  -9.809 -19.422  1.00 36.76   6        C
ATOM   2978  OG  SER B 166     -17.986 -10.287 -18.396  1.00 37.78   8        O
ATOM   2979  C   SER B 166     -14.885  -9.027 -20.076  1.00 35.94   6        C
ATOM   2980  O   SER B 166     -14.591  -9.721 -21.036  1.00 35.60   8        O
ATOM   2981  N   ALA B 167     -14.525  -7.751 -19.986  1.00 35.33   7        N
ATOM   2982  CA  ALA B 167     -13.796  -7.124 -21.082  1.00 35.07   6        C
ATOM   2983  CB  ALA B 167     -14.776  -6.801 -22.223  1.00 35.05   6        C
ATOM   2984  C   ALA B 167     -13.039  -5.862 -20.692  1.00 34.47   6        C
ATOM   2985  O   ALA B 167     -13.225  -5.328 -19.607  1.00 34.75   8        O
ATOM   2986  N   ILE B 168     -12.165  -5.411 -21.585  1.00 34.39   7        N
ATOM   2987  CA  ILE B 168     -11.539  -4.101 -21.450  1.00 34.20   6        C
ATOM   2988  CB  ILE B 168      -9.998  -4.158 -21.534  1.00 34.23   6        C
ATOM   2989  CG1 ILE B 168      -9.404  -2.744 -21.420  1.00 34.54   6        C
ATOM   2990  CD1 ILE B 168      -7.881  -2.715 -21.338  1.00 36.87   6        C
ATOM   2991  CG2 ILE B 168      -9.533  -4.816 -22.826  1.00 34.54   6        C
ATOM   2992  C   ILE B 168     -12.127  -3.246 -22.574  1.00 34.13   6        C
ATOM   2993  O   ILE B 168     -12.311  -3.739 -23.698  1.00 33.84   8        O
ATOM   2994  N   ILE B 169     -12.468  -1.997 -22.249  1.00 33.64   7        N
ATOM   2995  CA  ILE B 169     -13.064  -1.057 -23.202  1.00 33.32   6        C
ATOM   2996  CB  ILE B 169     -14.420  -0.521 -22.676  1.00 33.61   6        C
ATOM   2997  CG1 ILE B 169     -15.469  -1.635 -22.639  1.00 33.72   6        C
ATOM   2998  CD1 ILE B 169     -15.300  -2.621 -21.494  1.00 36.49   6        C
ATOM   2999  CG2 ILE B 169     -14.922   0.618 -23.555  1.00 33.32   6        C
ATOM   3000  C   ILE B 169     -12.099   0.095 -23.474  1.00 33.28   6        C
ATOM   3001  O   ILE B 169     -11.649   0.774 -22.552  1.00 33.30   8        O
ATOM   3002  N   LEU B 170     -11.765   0.292 -24.740  1.00 33.25   7        N
ATOM   3003  CA  LEU B 170     -10.801   1.310 -25.137  1.00 33.87   6        C
ATOM   3004  CB  LEU B 170      -9.625   0.642 -25.845  1.00 33.84   6        C
ATOM   3005  CG  LEU B 170      -8.876  -0.317 -24.920  1.00 34.72   6        C
ATOM   3006  CD1 LEU B 170      -8.437  -1.581 -25.646  1.00 36.16   6        C
ATOM   3007  CD2 LEU B 170      -7.686   0.400 -24.301  1.00 35.50   6        C
ATOM   3008  C   LEU B 170     -11.434   2.353 -26.043  1.00 34.26   6        C
ATOM   3009  O   LEU B 170     -11.923   2.033 -27.118  1.00 33.92   8        O
ATOM   3010  N   HIS B 171     -11.406   3.606 -25.602  1.00 35.06   7        N
ATOM   3011  CA  HIS B 171     -11.995   4.704 -26.364  1.00 35.98   6        C
ATOM   3012  CB  HIS B 171     -12.955   5.487 -25.460  1.00 35.84   6        C
```

```
ATOM   3013  CG  HIS B 171     -13.669   6.609 -26.147  1.00 36.49      6       C
ATOM   3014  ND1 HIS B 171     -14.038   6.562 -27.473  1.00 37.25      7       N
ATOM   3015  CE1 HIS B 171     -14.645   7.690 -27.800  1.00 37.12      6       C
ATOM   3016  NE2 HIS B 171     -14.690   8.463 -26.731  1.00 36.55      7       N
ATOM   3017  CD2 HIS B 171     -14.087   7.811 -25.683  1.00 36.20      6       C
ATOM   3018  C   HIS B 171     -10.918   5.622 -26.955  1.00 36.33      6       C
ATOM   3019  O   HIS B 171     -10.269   6.368 -26.227  1.00 36.67      8       O
ATOM   3020  N   LEU B 172     -10.742   5.561 -28.274  1.00 36.92      7       N
ATOM   3021  CA  LEU B 172      -9.744   6.368 -28.976  1.00 37.64      6       C
ATOM   3022  CB  LEU B 172      -9.212   5.612 -30.201  1.00 37.85      6       C
ATOM   3023  CG  LEU B 172      -8.422   4.324 -29.951  1.00 37.61      6       C
ATOM   3024  CD1 LEU B 172      -8.052   3.648 -31.266  1.00 38.15      6       C
ATOM   3025  CD2 LEU B 172      -7.169   4.620 -29.132  1.00 38.00      6       C
ATOM   3026  C   LEU B 172     -10.285   7.735 -29.400  1.00 38.28      6       C
ATOM   3027  O   LEU B 172     -11.499   7.924 -29.529  1.00 38.09      8       O
ATOM   3028  N   SER B 173      -9.372   8.675 -29.642  1.00 38.69      7       N
ATOM   3029  CA  SER B 173      -9.727  10.056 -29.982  1.00 39.00      6       C
ATOM   3030  CB  SER B 173      -8.496  10.959 -29.858  1.00 39.22      6       C
ATOM   3031  OG  SER B 173      -7.450  10.474 -30.683  1.00 38.70      8       O
ATOM   3032  C   SER B 173     -10.358  10.237 -31.360  1.00 39.22      6       C
ATOM   3033  O   SER B 173     -10.952  11.283 -31.639  1.00 39.75      8       O
ATOM   3034  N   ASN B 174     -10.222   9.241 -32.231  1.00 38.69      7       N
ATOM   3035  CA  ASN B 174     -10.866   9.325 -33.533  1.00 38.56      6       C
ATOM   3036  CB  ASN B 174     -10.104   8.543 -34.607  1.00 38.62      6       C
ATOM   3037  CG  ASN B 174     -10.080   7.046 -34.351  1.00 39.22      6       C
ATOM   3038  OD1 ASN B 174     -10.567   6.561 -33.322  1.00 39.24      8       O
ATOM   3039  ND2 ASN B 174      -9.499   6.302 -35.293  1.00 37.86      7       N
ATOM   3040  C   ASN B 174     -12.323   8.874 -33.439  1.00 37.88      6       C
ATOM   3041  O   ASN B 174     -13.026   8.800 -34.443  1.00 37.60      8       O
ATOM   3042  N   GLY B 175     -12.754   8.565 -32.220  1.00 37.14      7       N
ATOM   3043  CA  GLY B 175     -14.126   8.163 -31.968  1.00 36.74      6       C
ATOM   3044  C   GLY B 175     -14.361   6.664 -31.880  1.00 36.07      6       C
ATOM   3045  O   GLY B 175     -15.415   6.231 -31.431  1.00 35.97      8       O
ATOM   3046  N   SER B 176     -13.386   5.871 -32.308  1.00 35.44      7       N
ATOM   3047  CA  SER B 176     -13.526   4.419 -32.266  1.00 34.98      6       C
ATOM   3048  CB  SER B 176     -12.402   3.737 -33.039  1.00 34.77      6       C
ATOM   3049  OG  SER B 176     -12.541   3.974 -34.423  1.00 35.05      8       O
ATOM   3050  C   SER B 176     -13.557   3.885 -30.847  1.00 34.41      6       C
ATOM   3051  O   SER B 176     -12.898   4.415 -29.954  1.00 34.86      8       O
ATOM   3052  N   VAL B 177     -14.339   2.832 -30.646  1.00 33.80      7       N
ATOM   3053  CA  VAL B 177     -14.424   2.178 -29.355  1.00 32.73      6       C
ATOM   3054  CB  VAL B 177     -15.810   2.340 -28.734  1.00 33.19      6       C
ATOM   3055  CG1 VAL B 177     -15.914   1.543 -27.446  1.00 32.36      6       C
ATOM   3056  CG2 VAL B 177     -16.107   3.823 -28.466  1.00 32.87      6       C
ATOM   3057  C   VAL B 177     -14.103   0.698 -29.568  1.00 32.66      6       C
ATOM   3058  O   VAL B 177     -14.716   0.032 -30.407  1.00 31.91      8       O
ATOM   3059  N   GLN B 178     -13.120   0.200 -28.829  1.00 31.88      7       N
ATOM   3060  CA  GLN B 178     -12.699  -1.185 -28.973  1.00 32.15      6       C
ATOM   3061  CB  GLN B 178     -11.198  -1.274 -29.260  1.00 32.48      6       C
ATOM   3062  CG  GLN B 178     -10.692  -2.708 -29.355  1.00 32.29      6       C
ATOM   3063  CD  GLN B 178      -9.298  -2.798 -29.915  1.00 32.59      6       C
ATOM   3064  OE1 GLN B 178      -8.862  -1.923 -30.671  1.00 32.25      8       O
ATOM   3065  NE2 GLN B 178      -8.589  -3.859 -29.552  1.00 33.06      7       N
ATOM   3066  C   GLN B 178     -13.029  -1.947 -27.715  1.00 31.55      6       C
ATOM   3067  O   GLN B 178     -12.812  -1.449 -26.609  1.00 31.51      8       O
ATOM   3068  N   ILE B 179     -13.587  -3.144 -27.885  1.00 31.35      7       N
ATOM   3069  CA  ILE B 179     -13.932  -3.995 -26.755  1.00 30.76      6       C
```

```
ATOM   3070  CB  ILE B 179     -15.466  -4.117 -26.602  1.00 30.79      6       C
ATOM   3071  CG1 ILE B 179     -16.121  -2.739 -26.491  1.00 30.81      6       C
ATOM   3072  CD1 ILE B 179     -17.647  -2.801 -26.483  1.00 31.41      6       C
ATOM   3073  CG2 ILE B 179     -15.828  -4.966 -25.389  1.00 30.53      6       C
ATOM   3074  C   ILE B 179     -13.299  -5.378 -26.934  1.00 31.25      6       C
ATOM   3075  O   ILE B 179     -13.595  -6.082 -27.909  1.00 30.19      8       O
ATOM   3076  N   ASN B 180     -12.419  -5.750 -26.004  1.00 31.44      7       N
ATOM   3077  CA  ASN B 180     -11.754  -7.059 -26.029  1.00 32.18      6       C
ATOM   3078  CB  ASN B 180     -10.245  -6.933 -25.787  1.00 32.08      6       C
ATOM   3079  CG  ASN B 180      -9.514  -6.306 -26.943  1.00 33.32      6       C
ATOM   3080  OD1 ASN B 180     -10.120  -5.723 -27.832  1.00 33.17      8       O
ATOM   3081  ND2 ASN B 180      -8.187  -6.419 -26.936  1.00 33.68      7       N
ATOM   3082  C   ASN B 180     -12.322  -7.922 -24.930  1.00 32.14      6       C
ATOM   3083  O   ASN B 180     -12.172  -7.597 -23.750  1.00 32.05      8       O
ATOM   3084  N   PHE B 181     -12.977  -9.016 -25.302  1.00 32.45      7       N
ATOM   3085  CA  PHE B 181     -13.565  -9.919 -24.318  1.00 32.92      6       C
ATOM   3086  CB  PHE B 181     -14.752 -10.685 -24.921  1.00 32.53      6       C
ATOM   3087  CG  PHE B 181     -15.944  -9.809 -25.216  1.00 33.45      6       C
ATOM   3088  CD1 PHE B 181     -16.174  -9.336 -26.498  1.00 31.84      6       C
ATOM   3089  CE1 PHE B 181     -17.263  -8.509 -26.770  1.00 32.71      6       C
ATOM   3090  CZ  PHE B 181     -18.130  -8.158 -25.757  1.00 31.84      6       C
ATOM   3091  CE2 PHE B 181     -17.904  -8.617 -24.474  1.00 32.79      6       C
ATOM   3092  CD2 PHE B 181     -16.815  -9.435 -24.204  1.00 32.67      6       C
ATOM   3093  C   PHE B 181     -12.505 -10.875 -23.766  1.00 33.64      6       C
ATOM   3094  O   PHE B 181     -11.829 -11.555 -24.525  1.00 33.85      8       O
ATOM   3095  N   PHE B 182     -12.376 -10.912 -22.442  1.00 34.71      7       N
ATOM   3096  CA  PHE B 182     -11.360 -11.722 -21.755  1.00 35.89      6       C
ATOM   3097  CB  PHE B 182     -11.391 -11.442 -20.245  1.00 35.83      6       C
ATOM   3098  CG  PHE B 182     -11.052 -10.023 -19.877  1.00 35.30      6       C
ATOM   3099  CD1 PHE B 182     -11.754  -9.373 -18.879  1.00 34.96      6       C
ATOM   3100  CE1 PHE B 182     -11.449  -8.081 -18.533  1.00 35.25      6       C
ATOM   3101  CZ  PHE B 182     -10.421  -7.413 -19.185  1.00 35.90      6       C
ATOM   3102  CE2 PHE B 182      -9.717  -8.045 -20.180  1.00 35.75      6       C
ATOM   3103  CD2 PHE B 182     -10.033  -9.348 -20.521  1.00 35.84      6       C
ATOM   3104  C   PHE B 182     -11.426 -13.235 -21.960  1.00 36.43      6       C
ATOM   3105  O   PHE B 182     -10.459 -13.841 -22.408  1.00 37.17      8       O
ATOM   3106  N   GLN B 183     -12.554 -13.847 -21.622  1.00 37.30      7       N
ATOM   3107  CA  GLN B 183     -12.657 -15.315 -21.637  1.00 37.89      6       C
ATOM   3108  CB  GLN B 183     -13.905 -15.795 -20.885  1.00 38.49      6       C
ATOM   3109  CG  GLN B 183     -13.600 -16.691 -19.676  1.00 42.03      6       C
ATOM   3110  CD  GLN B 183     -14.591 -17.834 -19.544  1.00 45.34      6       C
ATOM   3111  OE1 GLN B 183     -15.503 -17.964 -20.365  1.00 47.51      8       O
ATOM   3112  NE2 GLN B 183     -14.417 -18.665 -18.516  1.00 46.40      7       N
ATOM   3113  C   GLN B 183     -12.576 -16.025 -22.988  1.00 37.30      6       C
ATOM   3114  O   GLN B 183     -11.961 -17.090 -23.093  1.00 36.80      8       O
ATOM   3115  N   ASP B 184     -13.191 -15.455 -24.019  1.00 36.51      7       N
ATOM   3116  CA  ASP B 184     -13.233 -16.129 -25.314  1.00 35.92      6       C
ATOM   3117  CB  ASP B 184     -14.667 -16.189 -25.830  1.00 36.35      6       C
ATOM   3118  CG  ASP B 184     -15.262 -14.814 -26.017  1.00 36.30      6       C
ATOM   3119  OD1 ASP B 184     -14.506 -13.900 -26.397  1.00 35.71      8       O
ATOM   3120  OD2 ASP B 184     -16.456 -14.545 -25.775  1.00 39.16      8       O
ATOM   3121  C   ASP B 184     -12.333 -15.493 -26.357  1.00 35.04      6       C
ATOM   3122  O   ASP B 184     -12.241 -15.984 -27.475  1.00 35.28      8       O
ATOM   3123  N   HIS B 185     -11.689 -14.388 -26.001  1.00 34.06      7       N
ATOM   3124  CA  HIS B 185     -10.746 -13.735 -26.902  1.00 33.53      6       C
ATOM   3125  CB  HIS B 185      -9.706 -14.749 -27.362  1.00 34.21      6       C
ATOM   3126  CG  HIS B 185      -8.925 -15.342 -26.232  1.00 36.01      6       C
```

ATOM	**3127**	**ND1**	**HIS**	**B**	**185**	**-8.240**	**-14.567**	**-25.321**	**1.00**	**37.51**	**7**	**N**
ATOM	**3128**	**CE1**	**HIS**	**B**	**185**	**-7.657**	**-15.349**	**-24.430**	**1.00**	**38.12**	**6**	**C**
ATOM	**3129**	**NE2**	**HIS**	**B**	**185**	**-7.946**	**-16.603**	**-24.726**	**1.00**	**38.61**	**7**	**N**
ATOM	**3130**	**CD2**	**HIS**	**B**	**185**	**-8.742**	**-16.627**	**-25.848**	**1.00**	**37.72**	**6**	**C**
ATOM	**3131**	**C**	**HIS**	**B**	**185**	**-11.354**	**-13.017**	**-28.121**	**1.00**	**32.46**	**6**	**C**
ATOM	**3132**	**O**	**HIS**	**B**	**185**	**-10.627**	**-12.603**	**-29.026**	**1.00**	**31.72**	**8**	**O**
ATOM	3133	N	THR	B	186	-12.674	-12.876	-28.149	1.00	31.83	7	N
ATOM	3134	CA	THR	B	186	-13.302	-12.146	-29.250	1.00	31.41	6	C
ATOM	3135	CB	THR	B	186	-14.777	-12.539	-29.430	1.00	31.18	6	C
ATOM	3136	OG1	THR	B	186	-15.477	-12.376	-28.196	1.00	30.58	8	O
ATOM	3137	CG2	THR	B	186	-14.921	-14.034	-29.737	1.00	31.70	6	C
ATOM	3138	C	THR	B	186	-13.178	-10.638	-29.001	1.00	31.34	6	C
ATOM	3139	O	THR	B	186	-13.050	-10.200	-27.855	1.00	31.21	8	O
ATOM	**3140**	**N**	**LYS**	**B**	**187**	**-13.212**	**-9.852**	**-30.075**	**1.00**	**30.85**	**7**	**N**
ATOM	**3141**	**CA**	**LYS**	**B**	**187**	**-13.076**	**-8.406**	**-29.958**	**1.00**	**30.83**	**6**	**C**
ATOM	**3142**	**CB**	**LYS**	**B**	**187**	**-11.626**	**-7.977**	**-30.210**	**1.00**	**31.19**	**6**	**C**
ATOM	**3143**	**CG**	**LYS**	**B**	**187**	**-10.576**	**-8.793**	**-29.461**	**1.00**	**31.58**	**6**	**C**
ATOM	**3144**	**CD**	**LYS**	**B**	**187**	**-9.185**	**-8.345**	**-29.866**	**1.00**	**34.32**	**6**	**C**
ATOM	**3145**	**CE**	**LYS**	**B**	**187**	**-8.109**	**-8.980**	**-28.981**	**1.00**	**34.39**	**6**	**C**
ATOM	**3146**	**NZ**	**LYS**	**B**	**187**	**-8.082**	**-10.456**	**-29.126**	**1.00**	**34.97**	**7**	**N**
ATOM	**3147**	**C**	**LYS**	**B**	**187**	**-13.961**	**-7.685**	**-30.966**	**1.00**	**30.47**	**6**	**C**
ATOM	**3148**	**O**	**LYS**	**B**	**187**	**-14.236**	**-8.211**	**-32.048**	**1.00**	**29.89**	**8**	**O**
ATOM	3149	N	LEU	B	188	-14.387	-6.480	-30.598	1.00	29.76	7	N
ATOM	3150	CA	LEU	B	188	-15.140	-5.618	-31.493	1.00	30.16	6	C
ATOM	3151	CB	LEU	B	188	-16.533	-5.320	-30.954	1.00	29.59	6	C
ATOM	3152	CG	LEU	B	188	-17.545	-6.424	-30.679	1.00	30.75	6	C
ATOM	3153	CD1	LEU	B	188	-18.701	-5.817	-29.923	1.00	31.35	6	C
ATOM	3154	CD2	LEU	B	188	-18.039	-7.056	-31.959	1.00	32.85	6	C
ATOM	3155	C	LEU	B	188	-14.406	-4.292	-31.639	1.00	30.15	6	C
ATOM	3156	O	LEU	B	188	-13.838	-3.775	-30.678	1.00	30.39	8	O
ATOM	3157	N	ILE	B	189	-14.404	-3.753	-32.845	1.00	30.49	7	N
ATOM	3158	CA	ILE	B	189	-13.876	-2.418	-33.076	1.00	30.85	6	C
ATOM	3159	CB	ILE	B	189	-12.663	-2.446	-34.002	1.00	30.93	6	C
ATOM	3160	CG1	ILE	B	189	-11.558	-3.346	-33.422	1.00	31.25	6	C
ATOM	3161	CD1	ILE	B	189	-10.629	-3.901	-34.473	1.00	33.02	6	C
ATOM	3162	CG2	ILE	B	189	-12.149	-1.025	-34.211	1.00	31.24	6	C
ATOM	3163	C	ILE	B	189	-15.010	-1.620	-33.712	1.00	31.21	6	C
ATOM	3164	O	ILE	B	189	-15.417	-1.912	-34.832	1.00	31.08	8	O
ATOM	3165	N	LEU	B	190	-15.527	-0.632	-32.988	1.00	31.34	7	N
ATOM	3166	CA	LEU	B	190	-16.656	0.159	-33.478	1.00	31.95	6	C
ATOM	3167	CB	LEU	B	190	-17.745	0.256	-32.404	1.00	31.74	6	C
ATOM	3168	CG	LEU	B	190	-18.423	-1.062	-31.992	1.00	31.40	6	C
ATOM	3169	CD1	LEU	B	190	-18.772	-1.078	-30.505	1.00	32.33	6	C
ATOM	3170	CD2	LEU	B	190	-19.668	-1.321	-32.823	1.00	30.49	6	C
ATOM	3171	C	LEU	B	190	-16.216	1.553	-33.908	1.00	32.26	6	C
ATOM	3172	O	LEU	B	190	-15.495	2.233	-33.187	1.00	32.36	8	O
ATOM	3173	N	CYS	B	191	-16.648	1.970	-35.093	1.00	32.91	7	N
ATOM	3174	CA	CYS	B	191	-16.347	3.310	-35.593	1.00	33.26	6	C
ATOM	3175	CB	CYS	B	191	-15.517	3.243	-36.873	1.00	33.24	6	C
ATOM	3176	SG	CYS	B	191	-15.232	4.869	-37.646	1.00	34.76	16	S
ATOM	3177	C	CYS	B	191	-17.657	4.032	-35.879	1.00	33.25	6	C
ATOM	3178	O	CYS	B	191	-18.436	3.575	-36.703	1.00	33.43	8	O
ATOM	3179	N	PRO	B	192	-17.904	5.141	-35.187	1.00	33.55	7	N
ATOM	3180	CA	PRO	B	192	-19.135	5.919	-35.361	1.00	34.06	6	C
ATOM	3181	CB	PRO	B	192	-19.162	6.758	-34.090	1.00	33.94	6	C
ATOM	3182	CG	PRO	B	192	-17.714	7.062	-33.888	1.00	33.71	6	C
ATOM	3183	CD	PRO	B	192	-17.042	5.729	-34.146	1.00	33.40	6	C

```
ATOM   3184  C   PRO B 192     -19.128   6.817 -36.600  1.00 34.90   6      C
ATOM   3185  O   PRO B 192     -20.185   7.339 -36.978  1.00 34.87   8      O
ATOM   3186  N   LEU B 193     -17.959   7.003 -37.212  1.00 35.55   7      N
ATOM   3187  CA  LEU B 193     -17.849   7.806 -38.429  1.00 36.44   6      C
ATOM   3188  CB  LEU B 193     -16.401   8.228 -38.670  1.00 36.83   6      C
ATOM   3189  CG  LEU B 193     -15.830   9.480 -38.002  1.00 38.55   6      C
ATOM   3190  CD1 LEU B 193     -16.082   9.509 -36.509  1.00 38.97   6      C
ATOM   3191  CD2 LEU B 193     -14.338   9.579 -38.290  1.00 39.57   6      C
ATOM   3192  C   LEU B 193     -18.339   6.986 -39.609  1.00 36.47   6      C
ATOM   3193  O   LEU B 193     -19.032   7.490 -40.488  1.00 37.34   8      O
ATOM   3194  N   MET B 194     -17.987   5.706 -39.617  1.00 36.22   7      N
ATOM   3195  CA  MET B 194     -18.396   4.804 -40.684  1.00 35.99   6      C
ATOM   3196  CB  MET B 194     -17.258   3.833 -41.006  1.00 36.75   6      C
ATOM   3197  CG  MET B 194     -15.974   4.507 -41.482  1.00 40.37   6      C
ATOM   3198  SD  MET B 194     -16.263   5.440 -42.975  1.00 48.21   16     S
ATOM   3199  CE  MET B 194     -16.542   4.127 -44.115  1.00 45.23   6      C
ATOM   3200  C   MET B 194     -19.637   4.001 -40.297  1.00 34.72   6      C
ATOM   3201  O   MET B 194     -20.161   3.232 -41.110  1.00 34.69   8      O
ATOM   3202  N   ALA B 195     -20.102   4.187 -39.061  1.00 32.98   7      N
ATOM   3203  CA  ALA B 195     -21.232   3.421 -38.532  1.00 31.57   6      C
ATOM   3204  CB  ALA B 195     -22.562   3.901 -39.143  1.00 31.57   6      C
ATOM   3205  C   ALA B 195     -21.001   1.938 -38.810  1.00 30.28   6      C
ATOM   3206  O   ALA B 195     -21.856   1.254 -39.373  1.00 29.74   8      O
ATOM   3207  N   ALA B 196     -19.836   1.447 -38.394  1.00 29.57   7      N
ATOM   3208  CA  ALA B 196     -19.434   0.076 -38.667  1.00 29.18   6      C
ATOM   3209  CB  ALA B 196     -18.423   0.049 -39.814  1.00 29.60   6      C
ATOM   3210  C   ALA B 196     -18.852  -0.646 -37.458  1.00 29.28   6      C
ATOM   3211  O   ALA B 196     -18.476  -0.023 -36.457  1.00 29.26   8      O
ATOM   3212  N   VAL B 197     -18.774  -1.965 -37.574  1.00 28.98   7      N
ATOM   3213  CA  VAL B 197     -18.202  -2.794 -36.526  1.00 28.77   6      C
ATOM   3214  CB  VAL B 197     -19.281  -3.482 -35.649  1.00 28.71   6      C
ATOM   3215  CG1 VAL B 197     -20.183  -4.404 -36.484  1.00 28.89   6      C
ATOM   3216  CG2 VAL B 197     -18.624  -4.270 -34.509  1.00 29.06   6      C
ATOM   3217  C   VAL B 197     -17.329  -3.851 -37.170  1.00 29.08   6      C
ATOM   3218  O   VAL B 197     -17.703  -4.458 -38.172  1.00 28.66   8      O
ATOM   3219  N   THR B 198     -16.147  -4.045 -36.599  1.00 29.05   7      N
ATOM   3220  CA  THR B 198     -15.260  -5.115 -37.017  1.00 29.47   6      C
ATOM   3221  CB  THR B 198     -13.830  -4.589 -37.150  1.00 29.50   6      C
ATOM   3222  OG1 THR B 198     -13.763  -3.713 -38.279  1.00 30.50   8      O
ATOM   3223  CG2 THR B 198     -12.858  -5.715 -37.527  1.00 29.77   6      C
ATOM   3224  C   THR B 198     -15.334  -6.159 -35.923  1.00 29.99   6      C
ATOM   3225  O   THR B 198     -15.176  -5.832 -34.745  1.00 29.34   8      O
ATOM   3226  N   TYR B 199     -15.615  -7.400 -36.308  1.00 29.93   7      N
ATOM   3227  CA  TYR B 199     -15.720  -8.472 -35.341  1.00 30.91   6      C
ATOM   3228  CB  TYR B 199     -17.046  -9.224 -35.521  1.00 30.44   6      C
ATOM   3229  CG  TYR B 199     -17.254 -10.364 -34.558  1.00 30.96   6      C
ATOM   3230  CD1 TYR B 199     -16.804 -10.285 -33.247  1.00 31.88   6      C
ATOM   3231  CE1 TYR B 199     -16.991 -11.325 -32.365  1.00 32.55   6      C
ATOM   3232  CZ  TYR B 199     -17.647 -12.459 -32.774  1.00 33.17   6      C
ATOM   3233  OH  TYR B 199     -17.840 -13.489 -31.878  1.00 36.31   8      O
ATOM   3234  CE2 TYR B 199     -18.110 -12.564 -34.067  1.00 33.56   6      C
ATOM   3235  CD2 TYR B 199     -17.915 -11.519 -34.952  1.00 32.17   6      C
ATOM   3236  C   TYR B 199     -14.554  -9.419 -35.560  1.00 31.41   6      C
ATOM   3237  O   TYR B 199     -14.332  -9.887 -36.673  1.00 31.16   8      O
ATOM   3238  N   ILE B 200     -13.794  -9.662 -34.502  1.00 32.55   7      N
ATOM   3239  CA  ILE B 200     -12.714 -10.635 -34.534  1.00 33.59   6      C
ATOM   3240  CB  ILE B 200     -11.414 -10.031 -33.962  1.00 33.63   6      C
```

```
ATOM   3241  CG1 ILE B 200     -10.948  -8.870 -34.843  1.00 33.74      6       C
ATOM   3242  CD1 ILE B 200      -9.712  -8.133 -34.330  1.00 35.27      6       C
ATOM   3243  CG2 ILE B 200     -10.325 -11.108 -33.866  1.00 33.81      6       C
ATOM   3244  C   ILE B 200     -13.198 -11.801 -33.693  1.00 34.45      6       C
ATOM   3245  O   ILE B 200     -13.412 -11.656 -32.496  1.00 34.78      8       O
ATOM   3246  N   ASP B 201     -13.410 -12.953 -34.318  1.00 35.60      7       N
ATOM   3247  CA  ASP B 201     -13.973 -14.086 -33.597  1.00 36.61      6       C
ATOM   3248  CB  ASP B 201     -14.868 -14.925 -34.508  1.00 36.72      6       C
ATOM   3249  CG  ASP B 201     -14.106 -15.575 -35.654  1.00 37.71      6       C
ATOM   3250  OD1 ASP B 201     -12.851 -15.613 -35.626  1.00 37.60      8       O
ATOM   3251  OD2 ASP B 201     -14.696 -16.088 -36.627  1.00 38.71      8       O
ATOM   3252  C   ASP B 201     -12.908 -14.948 -32.924  1.00 37.15      6       C
ATOM   3253  O   ASP B 201     -11.729 -14.620 -32.962  1.00 37.08      8       O
ATOM   3254  N   GLU B 202     -13.335 -16.045 -32.311  1.00 38.32      7       N
ATOM   3255  CA  GLU B 202     -12.408 -16.924 -31.599  1.00 39.84      6       C
ATOM   3256  CB  GLU B 202     -13.148 -17.883 -30.658  1.00 40.25      6       C
ATOM   3257  CG  GLU B 202     -14.367 -18.567 -31.256  1.00 42.60      6       C
ATOM   3258  CD  GLU B 202     -15.614 -17.703 -31.162  1.00 45.06      6       C
ATOM   3259  OE1 GLU B 202     -16.208 -17.626 -30.059  1.00 46.48      8       O
ATOM   3260  OE2 GLU B 202     -15.989 -17.097 -32.186  1.00 44.47      8       O
ATOM   3261  C   GLU B 202     -11.434 -17.690 -32.503  1.00 40.07      6       C
ATOM   3262  O   GLU B 202     -10.446 -18.235 -32.013  1.00 40.52      8       O
ATOM   3263  N   LYS B 203     -11.700 -17.730 -33.808  1.00 40.52      7       N
ATOM   3264  CA  LYS B 203     -10.784 -18.372 -34.754  1.00 41.15      6       C
ATOM   3265  CB  LYS B 203     -11.518 -18.902 -35.986  1.00 41.26      6       C
ATOM   3266  CG  LYS B 203     -12.568 -19.953 -35.768  1.00 42.20      6       C
ATOM   3267  CD  LYS B 203     -13.049 -20.405 -37.141  1.00 43.91      6       C
ATOM   3268  CE  LYS B 203     -14.421 -21.032 -37.111  1.00 45.01      6       C
ATOM   3269  NZ  LYS B 203     -14.983 -21.106 -38.487  1.00 45.80      7       N
ATOM   3270  C   LYS B 203      -9.806 -17.329 -35.254  1.00 41.28      6       C
ATOM   3271  O   LYS B 203      -8.943 -17.619 -36.080  1.00 41.11      8       O
ATOM   3272  N   ARG B 204      -9.971 -16.106 -34.761  1.00 41.53      7       N
ATOM   3273  CA  ARG B 204      -9.168 -14.958 -35.179  1.00 42.04      6       C
ATOM   3274  CB  ARG B 204      -7.666 -15.235 -35.094  1.00 42.26      6       C
ATOM   3275  CG  ARG B 204      -7.154 -15.387 -33.682  1.00 44.06      6       C
ATOM   3276  CD  ARG B 204      -5.672 -15.068 -33.530  1.00 47.27      6       C
ATOM   3277  NE  ARG B 204      -5.037 -15.848 -32.470  1.00 49.18      7       N
ATOM   3278  CZ  ARG B 204      -5.371 -15.794 -31.189  1.00 50.09      6       C
ATOM   3279  NH1 ARG B 204      -6.347 -14.989 -30.787  1.00 51.10      7       N
ATOM   3280  NH2 ARG B 204      -4.729 -16.549 -30.302  1.00 50.21      7       N
ATOM   3281  C   ARG B 204      -9.552 -14.443 -36.564  1.00 41.94      6       C
ATOM   3282  O   ARG B 204      -8.838 -13.641 -37.163  1.00 41.39      8       O
ATOM   3283  N   ASP B 205     -10.680 -14.912 -37.082  1.00 42.30      7       N
ATOM   3284  CA  ASP B 205     -11.159 -14.383 -38.343  1.00 42.63      6       C
ATOM   3285  CB  ASP B 205     -12.084 -15.355 -39.059  1.00 43.15      6       C
ATOM   3286  CG  ASP B 205     -11.363 -16.147 -40.116  1.00 45.20      6       C
ATOM   3287  OD1 ASP B 205     -11.487 -15.791 -41.308  1.00 48.54      8       O
ATOM   3288  OD2 ASP B 205     -10.636 -17.126 -39.847  1.00 46.34      8       O
ATOM   3289  C   ASP B 205     -11.847 -13.064 -38.078  1.00 42.42      6       C
ATOM   3290  O   ASP B 205     -12.376 -12.828 -36.988  1.00 42.54      8       O
ATOM   3291  N   PHE B 206     -11.827 -12.202 -39.080  1.00 41.94      7       N
ATOM   3292  CA  PHE B 206     -12.350 -10.863 -38.928  1.00 41.63      6       C
ATOM   3293  CB  PHE B 206     -11.191  -9.880 -38.826  1.00 41.67      6       C
ATOM   3294  CG  PHE B 206     -10.306  -9.887 -40.032  1.00 43.86      6       C
ATOM   3295  CD1 PHE B 206     -10.387  -8.874 -40.974  1.00 45.15      6       C
ATOM   3296  CE1 PHE B 206      -9.581  -8.890 -42.094  1.00 45.57      6       C
ATOM   3297  CZ  PHE B 206      -8.691  -9.931 -42.294  1.00 46.16      6       C
```

```
ATOM   3298  CE2 PHE B 206      -8.608 -10.952 -41.367  1.00 46.38    6        C
ATOM   3299  CD2 PHE B 206      -9.416 -10.929 -40.245  1.00 45.29    6        C
ATOM   3300  C   PHE B 206     -13.214 -10.475 -40.111  1.00 40.74    6        C
ATOM   3301  O   PHE B 206     -12.981 -10.896 -41.253  1.00 40.86    8        O
ATOM   3302  N   ARG B 207     -14.203  -9.644 -39.828  1.00 39.32    7        N
ATOM   3303  CA  ARG B 207     -15.099  -9.141 -40.843  1.00 38.06    6        C
ATOM   3304  CB  ARG B 207     -16.283 -10.086 -41.014  1.00 38.93    6        C
ATOM   3305  CG  ARG B 207     -15.921 -11.425 -41.649  1.00 41.23    6        C
ATOM   3306  CD  ARG B 207     -15.806 -11.365 -43.161  1.00 45.10    6        C
ATOM   3307  NE  ARG B 207     -15.000 -12.445 -43.720  1.00 47.95    7        N
ATOM   3308  CZ  ARG B 207     -15.354 -13.725 -43.735  1.00 49.42    6        C
ATOM   3309  NH1 ARG B 207     -16.504 -14.113 -43.204  1.00 50.39    7        N
ATOM   3310  NH2 ARG B 207     -14.548 -14.624 -44.281  1.00 49.46    7        N
ATOM   3311  C   ARG B 207     -15.575  -7.786 -40.365  1.00 36.54    6        C
ATOM   3312  O   ARG B 207     -15.753  -7.576 -39.167  1.00 35.56    8        O
ATOM   3313  N   THR B 208     -15.750  -6.864 -41.299  1.00 34.48    7        N
ATOM   3314  CA  THR B 208     -16.203  -5.526 -40.975  1.00 33.17    6        C
ATOM   3315  CB  THR B 208     -15.233  -4.497 -41.561  1.00 33.38    6        C
ATOM   3316  OG1 THR B 208     -13.963  -4.594 -40.887  1.00 32.93    8        O
ATOM   3317  CG2 THR B 208     -15.715  -3.091 -41.253  1.00 33.01    6        C
ATOM   3318  C   THR B 208     -17.590  -5.349 -41.577  1.00 32.35    6        C
ATOM   3319  O   THR B 208     -17.776  -5.583 -42.777  1.00 32.13    8        O
ATOM   3320  N   TYR B 209     -18.547  -4.929 -40.751  1.00 30.97    7        N
ATOM   3321  CA  TYR B 209     -19.938  -4.776 -41.175  1.00 30.20    6        C
ATOM   3322  CB  TYR B 209     -20.846  -5.716 -40.362  1.00 29.85    6        C
ATOM   3323  CG  TYR B 209     -20.446  -7.168 -40.380  1.00 30.61    6        C
ATOM   3324  CD1 TYR B 209     -19.672  -7.719 -39.351  1.00 30.41    6        C
ATOM   3325  CE1 TYR B 209     -19.302  -9.053 -39.377  1.00 31.94    6        C
ATOM   3326  CZ  TYR B 209     -19.718  -9.844 -40.433  1.00 31.53    6        C
ATOM   3327  OH  TYR B 209     -19.368 -11.171 -40.498  1.00 30.94    8        O
ATOM   3328  CE2 TYR B 209     -20.476  -9.310 -41.455  1.00 31.95    6        C
ATOM   3329  CD2 TYR B 209     -20.830  -7.989 -41.424  1.00 31.27    6        C
ATOM   3330  C   TYR B 209     -20.480  -3.377 -40.969  1.00 29.27    6        C
ATOM   3331  O   TYR B 209     -20.145  -2.726 -39.989  1.00 29.32    8        O
ATOM   3332  N   ARG B 210     -21.345  -2.925 -41.878  1.00 28.16    7        N
ATOM   3333  CA  ARG B 210     -22.070  -1.676 -41.658  1.00 27.88    6        C
ATOM   3334  CB  ARG B 210     -22.643  -1.138 -42.969  1.00 27.74    6        C
ATOM   3335  CG  ARG B 210     -21.594  -0.722 -43.996  1.00 29.12    6        C
ATOM   3336  CD  ARG B 210     -20.835   0.545 -43.644  1.00 30.87    6        C
ATOM   3337  NE  ARG B 210     -20.084   1.055 -44.796  1.00 30.84    7        N
ATOM   3338  CZ  ARG B 210     -19.609   2.288 -44.889  1.00 31.64    6        C
ATOM   3339  NH1 ARG B 210     -19.792   3.152 -43.899  1.00 31.10    7        N
ATOM   3340  NH2 ARG B 210     -18.953   2.671 -45.983  1.00 31.71    7        N
ATOM   3341  C   ARG B 210     -23.216  -1.973 -40.701  1.00 27.62    6        C
ATOM   3342  O   ARG B 210     -24.034  -2.859 -40.967  1.00 27.36    8        O
ATOM   3343  N   LEU B 211     -23.311  -1.219 -39.609  1.00 27.26    7        N
ATOM   3344  CA  LEU B 211     -24.358  -1.463 -38.613  1.00 27.06    6        C
ATOM   3345  CB  LEU B 211     -24.214  -0.482 -37.445  1.00 26.85    6        C
ATOM   3346  CG  LEU B 211     -22.968  -0.712 -36.575  1.00 26.35    6        C
ATOM   3347  CD1 LEU B 211     -22.809   0.428 -35.570  1.00 27.27    6        C
ATOM   3348  CD2 LEU B 211     -23.061  -2.065 -35.860  1.00 27.54    6        C
ATOM   3349  C   LEU B 211     -25.774  -1.379 -39.201  1.00 27.36    6        C
ATOM   3350  O   LEU B 211     -26.637  -2.200 -38.891  1.00 26.70    8        O
ATOM   3351  N   SER B 212     -26.013  -0.377 -40.039  1.00 27.73    7        N
ATOM   3352  CA  SER B 212     -27.332  -0.234 -40.668  1.00 28.63    6        C
ATOM   3353  CB  SER B 212     -27.441   1.079 -41.449  1.00 28.91    6        C
ATOM   3354  OG  SER B 212     -27.316   2.220 -40.608  1.00 32.24    8        O
```

```
ATOM   3355  C   SER B 212     -27.653  -1.420 -41.582  1.00 28.29   6        C
ATOM   3356  O   SER B 212     -28.819  -1.789 -41.763  1.00 28.79   8        O
ATOM   3357  N   LEU B 213     -26.624  -2.010 -42.183  1.00 28.05   7        N
ATOM   3358  CA  LEU B 213     -26.835  -3.180 -43.029  1.00 27.68   6        C
ATOM   3359  CB  LEU B 213     -25.668  -3.372 -43.994  1.00 27.38   6        C
ATOM   3360  CG  LEU B 213     -25.551  -2.331 -45.113  1.00 26.68   6        C
ATOM   3361  CD1 LEU B 213     -24.458  -2.754 -46.068  1.00 26.26   6        C
ATOM   3362  CD2 LEU B 213     -26.893  -2.195 -45.857  1.00 26.57   6        C
ATOM   3363  C   LEU B 213     -27.095  -4.462 -42.216  1.00 28.03   6        C
ATOM   3364  O   LEU B 213     -27.747  -5.397 -42.695  1.00 27.81   8        O
ATOM   3365  N   LEU B 214     -26.560  -4.532 -41.001  1.00 27.99   7        N
ATOM   3366  CA  LEU B 214     -26.835  -5.696 -40.163  1.00 28.19   6        C
ATOM   3367  CB  LEU B 214     -25.940  -5.723 -38.914  1.00 27.94   6        C
ATOM   3368  CG  LEU B 214     -24.447  -6.003 -39.141  1.00 28.61   6        C
ATOM   3369  CD1 LEU B 214     -23.665  -5.834 -37.824  1.00 29.52   6        C
ATOM   3370  CD2 LEU B 214     -24.214  -7.392 -39.722  1.00 28.82   6        C
ATOM   3371  C   LEU B 214     -28.312  -5.679 -39.788  1.00 28.08   6        C
ATOM   3372  O   LEU B 214     -28.947  -6.720 -39.636  1.00 28.21   8        O
ATOM   3373  N   GLU B 215     -28.858  -4.476 -39.673  1.00 29.11   7        N
ATOM   3374  CA  GLU B 215     -30.260  -4.263 -39.352  1.00 30.18   6        C
ATOM   3375  CB  GLU B 215     -30.453  -2.758 -39.187  1.00 30.65   6        C
ATOM   3376  CG  GLU B 215     -31.844  -2.267 -38.869  1.00 33.74   6        C
ATOM   3377  CD  GLU B 215     -31.793  -0.878 -38.253  1.00 37.26   6        C
ATOM   3378  OE1 GLU B 215     -30.680  -0.444 -37.846  1.00 38.83   8        O
ATOM   3379  OE2 GLU B 215     -32.852  -0.223 -38.186  1.00 39.07   8        O
ATOM   3380  C   GLU B 215     -31.190  -4.810 -40.442  1.00 30.23   6        C
ATOM   3381  O   GLU B 215     -32.238  -5.425 -40.171  1.00 30.85   8        O
ATOM   3382  N   GLU B 216     -30.792  -4.601 -41.685  1.00 29.38   7        N
ATOM   3383  CA  GLU B 216     -31.588  -5.031 -42.815  1.00 29.69   6        C
ATOM   3384  CB  GLU B 216     -31.238  -4.165 -44.036  1.00 29.56   6        C
ATOM   3385  CG  GLU B 216     -31.732  -2.726 -43.962  1.00 31.01   6        C
ATOM   3386  CD  GLU B 216     -33.244  -2.621 -43.913  1.00 32.22   6        C
ATOM   3387  OE1 GLU B 216     -33.815  -2.644 -42.796  1.00 35.17   8        O
ATOM   3388  OE2 GLU B 216     -33.867  -2.525 -44.989  1.00 32.31   8        O
ATOM   3389  C   GLU B 216     -31.405  -6.514 -43.155  1.00 29.31   6        C
ATOM   3390  O   GLU B 216     -32.372  -7.203 -43.479  1.00 30.41   8        O
ATOM   3391  N   TYR B 217     -30.180  -7.013 -43.056  1.00 29.30   7        N
ATOM   3392  CA  TYR B 217     -29.882  -8.370 -43.499  1.00 29.29   6        C
ATOM   3393  CB  TYR B 217     -28.592  -8.380 -44.321  1.00 29.39   6        C
ATOM   3394  CG  TYR B 217     -28.745  -7.699 -45.664  1.00 29.10   6        C
ATOM   3395  CD1 TYR B 217     -28.194  -6.449 -45.891  1.00 29.63   6        C
ATOM   3396  CE1 TYR B 217     -28.339  -5.814 -47.126  1.00 29.43   6        C
ATOM   3397  CZ  TYR B 217     -29.042  -6.431 -48.146  1.00 31.15   6        C
ATOM   3398  OH  TYR B 217     -29.169  -5.788 -49.369  1.00 31.03   8        O
ATOM   3399  CE2 TYR B 217     -29.604  -7.672 -47.947  1.00 31.19   6        C
ATOM   3400  CD2 TYR B 217     -29.461  -8.301 -46.699  1.00 30.94   6        C
ATOM   3401  C   TYR B 217     -29.807  -9.420 -42.399  1.00 29.70   6        C
ATOM   3402  O   TYR B 217     -29.875 -10.616 -42.678  1.00 29.44   8        O
ATOM   3403  N   GLY B 218     -29.648  -8.961 -41.163  1.00 30.30   7        N
ATOM   3404  CA  GLY B 218     -29.556  -9.833 -40.008  1.00 30.67   6        C
ATOM   3405  C   GLY B 218     -28.126 -10.233 -39.703  1.00 31.35   6        C
ATOM   3406  O   GLY B 218     -27.209  -9.917 -40.466  1.00 30.66   8        O
ATOM   3407  N   CYS B 219     -27.930 -10.913 -38.572  1.00 31.71   7        N
ATOM   3408  CA  CYS B 219     -26.612 -11.439 -38.224  1.00 32.39   6        C
ATOM   3409  CB  CYS B 219     -25.646 -10.339 -37.797  1.00 32.73   6        C
ATOM   3410  SG  CYS B 219     -25.858  -9.654 -36.140  1.00 34.72  16        S
ATOM   3411  C   CYS B 219     -26.697 -12.566 -37.193  1.00 32.51   6        C
```

ATOM	3412	O	CYS	B	219	-27.754	-12.803	-36.628	1.00	32.57	8	O
ATOM	3413	N	CYS	B	220	-25.587	-13.262	-36.970	1.00	32.50	7	N
ATOM	3414	CA	CYS	B	220	-25.554	-14.380	-36.027	1.00	32.97	6	C
ATOM	3415	CB	CYS	B	220	-24.231	-15.146	-36.150	1.00	33.12	6	C
ATOM	3416	SG	CYS	B	220	-22.768	-14.149	-35.739	1.00	37.64	16	S
ATOM	3417	C	CYS	B	220	-25.690	-13.925	-34.584	1.00	32.49	6	C
ATOM	3418	O	CYS	B	220	-25.447	-12.759	-34.262	1.00	31.34	8	O
ATOM	3419	N	LYS	B	221	-26.046	-14.871	-33.717	1.00	32.02	7	N
ATOM	3420	CA	LYS	B	221	-26.174	-14.607	-32.286	1.00	32.77	6	C
ATOM	3421	CB	LYS	B	221	-26.625	-15.876	-31.552	1.00	32.93	6	C
ATOM	3422	CG	LYS	B	221	-28.120	-16.043	-31.463	1.00	36.27	6	C
ATOM	3423	CD	LYS	B	221	-28.473	-17.215	-30.528	1.00	39.66	6	C
ATOM	3424	CE	LYS	B	221	-29.913	-17.111	-30.032	1.00	41.43	6	C
ATOM	3425	NZ	LYS	B	221	-30.246	-18.141	-29.000	1.00	43.73	7	N
ATOM	3426	C	LYS	B	221	-24.868	-14.109	-31.674	1.00	32.01	6	C
ATOM	3427	O	LYS	B	221	-24.887	-13.267	-30.777	1.00	32.02	8	O
ATOM	3428	N	GLU	B	222	-23.749	-14.666	-32.136	1.00	31.92	7	N
ATOM	3429	CA	GLU	B	222	-22.404	-14.273	-31.700	1.00	31.80	6	C
ATOM	3430	CB	GLU	B	222	-21.364	-14.845	-32.672	1.00	32.80	6	C
ATOM	3431	CG	GLU	B	222	-20.442	-15.939	-32.158	1.00	37.22	6	C
ATOM	3432	CD	GLU	B	222	-19.162	-16.024	-32.990	1.00	41.59	6	C
ATOM	3433	OE1	GLU	B	222	-18.071	-15.787	-32.426	1.00	42.25	8	O
ATOM	3434	OE2	GLU	B	222	-19.245	-16.296	-34.220	1.00	44.47	8	O
ATOM	3435	C	GLU	B	222	-22.222	-12.758	-31.725	1.00	30.78	6	C
ATOM	3436	O	GLU	B	222	-21.904	-12.116	-30.715	1.00	30.00	8	O
ATOM	3437	N	LEU	B	223	-22.388	-12.189	-32.913	1.00	29.53	7	N
ATOM	3438	CA	LEU	B	223	-22.209	-10.757	-33.092	1.00	28.77	6	C
ATOM	3439	CB	LEU	B	223	-22.097	-10.417	-34.586	1.00	29.52	6	C
ATOM	3440	CG	LEU	B	223	-21.901	-8.933	-34.861	1.00	30.50	6	C
ATOM	3441	CD1	LEU	B	223	-20.755	-8.407	-34.013	1.00	31.04	6	C
ATOM	3442	CD2	LEU	B	223	-21.651	-8.685	-36.358	1.00	32.50	6	C
ATOM	3443	C	LEU	B	223	-23.330	-9.950	-32.453	1.00	28.10	6	C
ATOM	3444	O	LEU	B	223	-23.075	-8.936	-31.809	1.00	27.14	8	O
ATOM	3445	N	ALA	B	224	-24.574	-10.394	-32.631	1.00	27.70	7	N
ATOM	3446	CA	ALA	B	224	-25.721	-9.676	-32.070	1.00	26.94	6	C
ATOM	3447	CB	ALA	B	224	-27.044	-10.393	-32.420	1.00	27.22	6	C
ATOM	3448	C	ALA	B	224	-25.609	-9.496	-30.556	1.00	27.06	6	C
ATOM	3449	O	ALA	B	224	-25.849	-8.408	-30.034	1.00	26.24	8	O
ATOM	3450	N	SER	B	225	-25.269	-10.575	-29.854	1.00	26.87	7	N
ATOM	3451	CA	SER	B	225	-25.155	-10.529	-28.399	1.00	27.50	6	C
ATOM	3452	CB	SER	B	225	-24.989	-11.945	-27.823	1.00	27.52	6	C
ATOM	3453	OG	SER	B	225	-23.738	-12.509	-28.196	1.00	29.93	8	O
ATOM	3454	C	SER	B	225	-24.023	-9.587	-27.964	1.00	27.29	6	C
ATOM	3455	O	SER	B	225	-24.152	-8.853	-26.979	1.00	27.90	8	O
ATOM	3456	N	ARG	B	226	-22.929	-9.563	-28.712	1.00	26.68	7	N
ATOM	3457	CA	ARG	B	226	-21.837	-8.660	-28.355	1.00	26.54	6	C
ATOM	3458	CB	ARG	B	226	-20.537	-9.070	-29.038	1.00	26.73	6	C
ATOM	3459	CG	ARG	B	226	-19.945	-10.333	-28.437	1.00	26.92	6	C
ATOM	3460	CD	ARG	B	226	-18.949	-11.028	-29.331	1.00	28.63	6	C
ATOM	3461	NE	ARG	B	226	-18.380	-12.205	-28.675	1.00	28.84	7	N
ATOM	3462	CZ	ARG	B	226	-19.015	-13.364	-28.558	1.00	30.18	6	C
ATOM	3463	NH1	ARG	B	226	-20.231	-13.511	-29.066	1.00	29.81	7	N
ATOM	3464	NH2	ARG	B	226	-18.428	-14.386	-27.938	1.00	30.49	7	N
ATOM	3465	C	ARG	B	226	-22.171	-7.188	-28.621	1.00	26.70	6	C
ATOM	3466	O	ARG	B	226	-21.670	-6.307	-27.917	1.00	26.13	8	O
ATOM	3467	N	LEU	B	227	-23.018	-6.932	-29.625	1.00	26.74	7	N
ATOM	3468	CA	LEU	B	227	-23.500	-5.573	-29.911	1.00	27.07	6	C

```
ATOM   3469  CB  LEU B 227     -24.206  -5.510 -31.277  1.00 27.06    6       C
ATOM   3470  CG  LEU B 227     -23.289  -5.649 -32.505  1.00 27.87    6       C
ATOM   3471  CD1 LEU B 227     -24.049  -5.763 -33.847  1.00 29.73    6       C
ATOM   3472  CD2 LEU B 227     -22.303  -4.492 -32.555  1.00 28.95    6       C
ATOM   3473  C   LEU B 227     -24.424  -5.054 -28.799  1.00 27.49    6       C
ATOM   3474  O   LEU B 227     -24.465  -3.852 -28.523  1.00 27.09    8       O
ATOM   3475  N   ARG B 228     -25.183  -5.960 -28.178  1.00 27.37    7       N
ATOM   3476  CA  ARG B 228     -26.016  -5.593 -27.045  1.00 27.38    6       C
ATOM   3477  CB  ARG B 228     -26.915  -6.751 -26.610  1.00 28.17    6       C
ATOM   3478  CG  ARG B 228     -28.199  -6.972 -27.404  1.00 28.52    6       C
ATOM   3479  CD  ARG B 228     -29.157  -7.933 -26.667  1.00 33.60    6       C
ATOM   3480  NE  ARG B 228     -28.917  -9.320 -27.044  1.00 36.16    7       N
ATOM   3481  CZ  ARG B 228     -28.486 -10.282 -26.252  1.00 38.65    6       C
ATOM   3482  NH1 ARG B 228     -28.239 -10.052 -24.961  1.00 42.93    7       N
ATOM   3483  NH2 ARG B 228     -28.316 -11.500 -26.753  1.00 36.19    7       N
ATOM   3484  C   ARG B 228     -25.111  -5.184 -25.875  1.00 27.29    6       C
ATOM   3485  O   ARG B 228     -25.394  -4.213 -25.187  1.00 26.61    8       O
ATOM   3486  N   TYR B 229     -24.031  -5.932 -25.643  1.00 26.96    7       N
ATOM   3487  CA  TYR B 229     -23.086  -5.588 -24.573  1.00 27.38    6       C
ATOM   3488  CB  TYR B 229     -22.025  -6.686 -24.396  1.00 27.60    6       C
ATOM   3489  CG  TYR B 229     -21.122  -6.500 -23.184  1.00 28.68    6       C
ATOM   3490  CD1 TYR B 229     -21.572  -6.796 -21.909  1.00 30.32    6       C
ATOM   3491  CE1 TYR B 229     -20.760  -6.625 -20.798  1.00 30.49    6       C
ATOM   3492  CZ  TYR B 229     -19.479  -6.162 -20.961  1.00 32.12    6       C
ATOM   3493  OH  TYR B 229     -18.669  -5.989 -19.858  1.00 33.30    8       O
ATOM   3494  CE2 TYR B 229     -19.007  -5.850 -22.218  1.00 31.11    6       C
ATOM   3495  CD2 TYR B 229     -19.832  -6.017 -23.321  1.00 30.01    6       C
ATOM   3496  C   TYR B 229     -22.410  -4.261 -24.906  1.00 26.96    6       C
ATOM   3497  O   TYR B 229     -22.198  -3.409 -24.031  1.00 27.00    8       O
ATOM   3498  N   ALA B 230     -22.070  -4.093 -26.178  1.00 26.19    7       N
ATOM   3499  CA  ALA B 230     -21.397  -2.879 -26.612  1.00 26.77    6       C
ATOM   3500  CB  ALA B 230     -21.096  -2.930 -28.094  1.00 25.91    6       C
ATOM   3501  C   ALA B 230     -22.232  -1.651 -26.285  1.00 26.84    6       C
ATOM   3502  O   ALA B 230     -21.705  -0.652 -25.830  1.00 27.61    8       O
ATOM   3503  N   ARG B 231     -23.533  -1:723 -26.523  1.00 27.30    7       N
ATOM   3504  CA  ARG B 231     -24.383  -0.574 -26.241  1.00 27.93    6       C
ATOM   3505  CB  ARG B 231     -25.831  -0.838 -26.671  1.00 27.44    6       C
ATOM   3506  CG  ARG B 231     -26.767   0.364 -26.489  1.00 28.31    6       C
ATOM   3507  CD  ARG B 231     -27.516   0.376 -25.153  1.00 29.17    6       C
ATOM   3508  NE  ARG B 231     -28.261   1.625 -24.958  1.00 31.21    7       N
ATOM   3509  CZ  ARG B 231     -29.409   1.917 -25.571  1.00 32.23    6       C
ATOM   3510  NH1 ARG B 231     -29.949   1.054 -26.419  1.00 32.15    7       N
ATOM   3511  NH2 ARG B 231     -30.017   3.079 -25.343  1.00 32.54    7       N
ATOM   3512  C   ARG B 231     -24.293  -0.226 -24.756  1.00 28.42    6       C
ATOM   3513  O   ARG B 231     -24.230   0.953 -24.382  1.00 28.53    8       O
ATOM   3514  N   THR B 232     -24.291  -1.249 -23.903  1.00 28.73    7       N
ATOM   3515  CA  THR B 232     -24.150  -1.006 -22.466  1.00 29.42    6       C
ATOM   3516  CB  THR B 232     -24.227  -2.319 -21.670  1.00 29.47    6       C
ATOM   3517  OG1 THR B 232     -25.451  -2.985 -21.987  1.00 29.28    8       O
ATOM   3518  CG2 THR B 232     -24.353  -2.026 -20.173  1.00 30.08    6       C
ATOM   3519  C   THR B 232     -22.855  -0.264 -22.141  1.00 29.68    6       C
ATOM   3520  O   THR B 232     -22.860   0.682 -21.341  1.00 29.95    8       O
ATOM   3521  N   MET B 233     -21.754  -0.687 -22.762  1.00 30.00    7       N
ATOM   3522  CA  MET B 233     -20.441  -0.069 -22.544  1.00 30.54    6       C
ATOM   3523  CB  MET B 233     -19.337  -0.872 -23.245  1.00 30.12    6       C
ATOM   3524  CG  MET B 233     -19.145  -2.306 -22.732  1.00 30.73    6       C
ATOM   3525  SD  MET B 233     -18.887  -2.407 -20.932  1.00 31.99   16       S
```

```
ATOM   3526  CE  MET B 233     -20.446  -2.980 -20.352  1.00 26.77    6        C
ATOM   3527  C   MET B 233     -20.402   1.387 -23.018  1.00 31.10    6        C
ATOM   3528  O   MET B 233     -19.817   2.257 -22.364  1.00 30.77    8        O
ATOM   3529  N   VAL B 234     -21.016   1.647 -24.166  1.00 31.50    7        N
ATOM   3530  CA  VAL B 234     -21.057   3.010 -24.691  1.00 32.56    6        C
ATOM   3531  CB  VAL B 234     -21.588   3.031 -26.133  1.00 32.17    6        C
ATOM   3532  CG1 VAL B 234     -21.888   4.459 -26.576  1.00 32.60    6        C
ATOM   3533  CG2 VAL B 234     -20.567   2.359 -27.047  1.00 31.40    6        C
ATOM   3534  C   VAL B 234     -21.859   3.927 -23.760  1.00 33.51    6        C
ATOM   3535  O   VAL B 234     -21.476   5.082 -23.522  1.00 33.37    8        O
ATOM   3536  N   ASP B 235     -22.952   3.403 -23.211  1.00 34.93    7        N
ATOM   3537  CA  ASP B 235     -23.728   4.142 -22.225  1.00 36.74    6        C
ATOM   3538  CB  ASP B 235     -24.939   3.335 -21.758  1.00 37.07    6        C
ATOM   3539  CG  ASP B 235     -26.181   3.614 -22.582  1.00 38.43    6        C
ATOM   3540  OD1 ASP B 235     -26.322   4.750 -23.090  1.00 39.46    8        O
ATOM   3541  OD2 ASP B 235     -27.080   2.768 -22.764  1.00 40.23    8        O
ATOM   3542  C   ASP B 235     -22.845   4.503 -21.028  1.00 37.73    6        C
ATOM   3543  O   ASP B 235     -22.965   5.594 -20.473  1.00 38.05    8        O
ATOM   3544  N   LYS B 236     -21.963   3.585 -20.630  1.00 38.45    7        N
ATOM   3545  CA  LYS B 236     -21.037   3.841 -19.525  1.00 39.37    6        C
ATOM   3546  CB  LYS B 236     -20.323   2.551 -19.090  1.00 39.23    6        C
ATOM   3547  CG  LYS B 236     -21.217   1.548 -18.366  1.00 39.71    6        C
ATOM   3548  CD  LYS B 236     -20.471   0.246 -18.082  1.00 41.45    6        C
ATOM   3549  CE  LYS B 236     -21.307  -0.722 -17.248  1.00 42.36    6        C
ATOM   3550  NZ  LYS B 236     -21.538  -0.227 -15.857  1.00 43.08    7        N
ATOM   3551  C   LYS B 236     -20.023   4.945 -19.872  1.00 40.08    6        C
ATOM   3552  O   LYS B 236     -19.752   5.822 -19.049  1.00 40.28    8        O
ATOM   3553  N   LEU B 237     -19.472   4.909 -21.083  1.00 41.07    7        N
ATOM   3554  CA  LEU B 237     -18.556   5.959 -21.531  1.00 42.34    6        C
ATOM   3555  CB  LEU B 237     -18.019   5.659 -22.930  1.00 41.72    6        C
ATOM   3556  CG  LEU B 237     -17.053   4.480 -23.116  1.00 41.42    6        C
ATOM   3557  CD1 LEU B 237     -16.740   4.272 -24.589  1.00 40.13    6        C
ATOM   3558  CD2 LEU B 237     -15.768   4.706 -22.325  1.00 40.29    6        C
ATOM   3559  C   LEU B 237     -19.270   7.315 -21.526  1.00 43.87    6        C
ATOM   3560  O   LEU B 237     -18.680   8.346 -21.188  1.00 43.65    8        O
ATOM   3561  N   LEU B 238     -20.543   7.303 -21.909  1.00 45.67    7        N
ATOM   3562  CA  LEU B 238     -21.360   8.516 -21.938  1.00 47.83    6        C
ATOM   3563  CB  LEU B 238     -22.634   8.268 -22.739  1.00 47.56    6        C
ATOM   3564  CG  LEU B 238     -22.472   8.426 -24.246  1.00 48.06    6        C
ATOM   3565  CD1 LEU B 238     -23.576   7.688 -24.991  1.00 48.48    6        C
ATOM   3566  CD2 LEU B 238     -22.452   9.905 -24.616  1.00 47.93    6        C
ATOM   3567  C   LEU B 238     -21.725   9.030 -20.549  1.00 49.45    6        C
ATOM   3568  O   LEU B 238     -21.847  10.240 -20.335  1.00 49.72    8        O
ATOM   3569  N   SER B 239     -21.908   8.110 -19.609  1.00 51.38    7        N
ATOM   3570  CA  SER B 239     -22.268   8.477 -18.245  1.00 53.19    6        C
ATOM   3571  CB  SER B 239     -22.356   7.233 -17.361  1.00 53.24    6        C
ATOM   3572  OG  SER B 239     -21.057   6.755 -17.034  1.00 54.23    8        O
ATOM   3573  C   SER B 239     -21.252   9.437 -17.649  1.00 54.22    6        C
ATOM   3574  O   SER B 239     -21.596  10.537 -17.217  1.00 54.39    8        O
ATOM   3575  N   SER B 240     -19.996   9.006 -17.635  1.00 55.60    7        N
ATOM   3576  CA  SER B 240     -18.910   9.784 -17.054  1.00 56.85    6        C
ATOM   3577  CB  SER B 240     -18.060   8.884 -16.162  1.00 56.87    6        C
ATOM   3578  OG  SER B 240     -17.359   7.932 -16.948  1.00 57.47    8        O
ATOM   3579  C   SER B 240     -18.017  10.383 -18.128  1.00 57.56    6        C
ATOM   3580  O   SER B 240     -16.807  10.141 -18.132  1.00 58.04    8        O
ATOM   3581  N   ALA B 241     -18.605  11.155 -19.039  1.00 58.16    7        N
ATOM   3582  CA  ALA B 241     -17.842  11.764 -20.125  1.00 58.82    6        C
```

```
ATOM   3583  CB  ALA B 241     -18.769  12.239 -21.236  1.00 58.78   6       C
ATOM   3584  C   ALA B 241     -16.964  12.912 -19.633  1.00 59.25   6       C
ATOM   3585  O   ALA B 241     -17.366  14.082 -19.667  1.00 59.83   8       O
ATOM   3586  OXT ALA B 241     -15.832  12.686 -19.195  1.00 59.46   8       O
ATOM   3587  N   PRO E   1      16.379  -7.591   9.788  1.00 40.91   7       N
ATOM   3588  CA  PRO E   1      15.544  -7.800   8.572  1.00 40.69   6       C
ATOM   3589  CB  PRO E   1      14.852  -6.442   8.381  1.00 40.92   6       C
ATOM   3590  CG  PRO E   1      15.200  -5.629   9.591  1.00 41.09   6       C
ATOM   3591  CD  PRO E   1      16.488  -6.166  10.134  1.00 41.02   6       C
ATOM   3592  C   PRO E   1      16.423  -8.073   7.359  1.00 40.59   6       C
ATOM   3593  O   PRO E   1      17.539  -7.559   7.287  1.00 40.41   8       O
ATOM   3594  N   MET E   2      15.918  -8.856   6.411  1.00 40.05   7       N
ATOM   3595  CA  MET E   2      16.683  -9.168   5.215  1.00 40.01   6       C
ATOM   3596  CB  MET E   2      16.476 -10.634   4.812  1.00 41.09   6       C
ATOM   3597  CG  MET E   2      17.149 -11.615   5.768  1.00 43.73   6       C
ATOM   3598  SD  MET E   2      18.945 -11.390   5.805  1.00 51.91  16       S
ATOM   3599  CE  MET E   2      19.424 -12.458   7.142  1.00 52.26   6       C
ATOM   3600  C   MET E   2      16.326  -8.218   4.083  1.00 38.83   6       C
ATOM   3601  O   MET E   2      16.804  -8.365   2.957  1.00 38.60   8       O
ATOM   3602  N   GLN E   3      15.486  -7.233   4.394  1.00 37.38   7       N
ATOM   3603  CA  GLN E   3      15.069  -6.232   3.427  1.00 36.61   6       C
ATOM   3604  CB  GLN E   3      13.811  -6.680   2.676  1.00 37.52   6       C
ATOM   3605  CG  GLN E   3      12.608  -7.020   3.564  1.00 39.75   6       C
ATOM   3606  CD  GLN E   3      11.396  -7.467   2.757  1.00 44.85   6       C
ATOM   3607  OE1 GLN E   3      11.509  -7.723   1.556  1.00 46.70   8       O
ATOM   3608  NE2 GLN E   3      10.235  -7.553   3.411  1.00 46.10   7       N
ATOM   3609  C   GLN E   3      14.793  -4.932   4.163  1.00 35.58   6       C
ATOM   3610  O   GLN E   3      14.584  -4.938   5.377  1.00 34.86   8       O
ATOM   3611  N   SER E   4      14.809  -3.821   3.437  1.00 34.23   7       N
ATOM   3612  CA  SER E   4      14.508  -2.524   4.040  1.00 33.40   6       C
ATOM   3613  CB  SER E   4      14.962  -1.393   3.125  1.00 32.85   6       C
ATOM   3614  OG  SER E   4      14.259  -1.436   1.895  1.00 31.74   8       O
ATOM   3615  C   SER E   4      13.003  -2.416   4.303  1.00 33.74   6       C
ATOM   3616  O   SER E   4      12.262  -3.389   4.107  1.00 33.39   8       O
ATOM   3617  O3P TPO E   5      13.899   1.162   8.266  1.00 30.51   8       O
ATOM   3618  P   TPO E   5      13.192   1.401   6.861  1.00 32.40  15       P
ATOM   3619  O1P TPO E   5      12.542   2.823   6.637  1.00 32.45   8       O
ATOM   3620  O2P TPO E   5      14.048   0.875   5.613  1.00 31.07   8       O
ATOM   3621  OG1 TPO E   5      11.927   0.412   6.990  1.00 32.05   8       O
ATOM   3622  CB  TPO E   5      11.038   0.274   5.883  1.00 33.24   6       C
ATOM   3623  CG2 TPO E   5       9.631   0.611   6.355  1.00 34.92   6       C
ATOM   3624  CA  TPO E   5      11.111  -1.171   5.347  1.00 33.88   6       C
ATOM   3625  N   TPO E   5      12.470  -1.314   4.833  1.00 33.34   7       N
ATOM   3626  C   TPO E   5      10.057  -1.420   4.285  1.00 34.38   6       C
ATOM   3627  O   TPO E   5      10.147  -0.852   3.087  1.00 33.69   8       O
ATOM   3628  N   PRO E   6       9.130  -2.342   4.537  1.00 38.71   7       N
ATOM   3629  CA  PRO E   6       8.008  -2.757   3.643  1.00 40.41   6       C
ATOM   3630  CB  PRO E   6       7.331  -3.894   4.422  1.00 40.16   6       C
ATOM   3631  CG  PRO E   6       8.323  -4.323   5.457  1.00 40.63   6       C
ATOM   3632  CD  PRO E   6       9.129  -3.091   5.804  1.00 39.21   6       C
ATOM   3633  C   PRO E   6       6.999  -1.642   3.392  1.00 41.29   6       C
ATOM   3634  O   PRO E   6       6.811  -0.739   4.215  1.00 41.42   8       O
ATOM   3635  N   LEU E   7       6.338  -1.742   2.247  1.00 42.62   7       N
ATOM   3636  CA  LEU E   7       5.340  -0.786   1.797  1.00 43.90   6       C
ATOM   3637  CB  LEU E   7       4.866  -1.200   0.403  1.00 44.21   6       C
ATOM   3638  CG  LEU E   7       4.188  -0.160  -0.479  1.00 45.75   6       C
ATOM   3639  CD1 LEU E   7       4.942   1.161  -0.420  1.00 46.28   6       C
```

```
ATOM   3640  CD2 LEU E   7       4.097  -0.682  -1.911  1.00 46.71   6       C
ATOM   3641  C   LEU E   7       4.152  -0.676   2.758  1.00 44.39   6       C
ATOM   3642  O   LEU E   7       3.923  -1.564   3.592  1.00 45.42   8       O
ATOM   3643  N   PRO F   1      -7.373  -9.873 -15.860  1.00 63.61   7       N
ATOM   3644  CA  PRO F   1      -6.089  -9.838 -16.612  1.00 63.48   6       C
ATOM   3645  CB  PRO F   1      -6.509 -10.235 -18.031  1.00 63.69   6       C
ATOM   3646  CG  PRO F   1      -7.815 -10.957 -17.863  1.00 63.62   6       C
ATOM   3647  CD  PRO F   1      -8.500 -10.293 -16.711  1.00 63.72   6       C
ATOM   3648  C   PRO F   1      -5.498  -8.433 -16.617  1.00 63.40   6       C
ATOM   3649  O   PRO F   1      -6.217  -7.470 -16.871  1.00 63.50   8       O
ATOM   3650  N   MET F   2      -4.204  -8.315 -16.343  1.00 63.09   7       N
ATOM   3651  CA  MET F   2      -3.557  -7.008 -16.314  1.00 62.84   6       C
ATOM   3652  CB  MET F   2      -2.636  -6.893 -15.099  1.00 63.11   6       C
ATOM   3653  CG  MET F   2      -3.383  -6.837 -13.779  1.00 64.18   6       C
ATOM   3654  SD  MET F   2      -4.393  -5.349 -13.628  1.00 66.44  16       S
ATOM   3655  CE  MET F   2      -5.583  -5.865 -12.403  1.00 66.00   6       C
ATOM   3656  C   MET F   2      -2.780  -6.745 -17.594  1.00 62.32   6       C
ATOM   3657  O   MET F   2      -2.021  -5.781 -17.685  1.00 62.29   8       O
ATOM   3658  N   GLN F   3      -2.984  -7.603 -18.585  1.00 61.72   7       N
ATOM   3659  CA  GLN F   3      -2.304  -7.475 -19.864  1.00 61.27   6       C
ATOM   3660  CB  GLN F   3      -0.896  -8.057 -19.762  1.00 61.46   6       C
ATOM   3661  CG  GLN F   3      -0.859  -9.414 -19.086  1.00 62.29   6       C
ATOM   3662  CD  GLN F   3       0.506 -10.055 -19.147  1.00 63.90   6       C
ATOM   3663  OE1 GLN F   3       1.511  -9.373 -19.352  1.00 64.45   8       O
ATOM   3664  NE2 GLN F   3       0.550 -11.371 -18.974  1.00 64.33   7       N
ATOM   3665  C   GLN F   3      -3.078  -8.213 -20.951  1.00 60.64   6       C
ATOM   3666  O   GLN F   3      -3.908  -9.072 -20.661  1.00 60.37   8       O
ATOM   3667  N   SER F   4      -2.802  -7.874 -22.204  1.00 60.11   7       N
ATOM   3668  CA  SER F   4      -3.463  -8.531 -23.324  1.00 59.74   6       C
ATOM   3669  CB  SER F   4      -3.501  -7.620 -24.550  1.00 59.65   6       C
ATOM   3670  OG  SER F   4      -2.201  -7.299 -25.014  1.00 59.69   8       O
ATOM   3671  C   SER F   4      -2.765  -9.846 -23.654  1.00 59.29   6       C
ATOM   3672  O   SER F   4      -2.174 -10.478 -22.776  1.00 59.28   8       O
ATOM   3673  O3P TPO F   5      -6.281 -11.938 -27.798  1.00 52.19   8       O
ATOM   3674  P   TPO F   5      -6.257 -11.464 -26.261  1.00 51.54  15       P
ATOM   3675  O1P TPO F   5      -5.611 -10.011 -26.100  1.00 51.18   8       O
ATOM   3676  O2P TPO F   5      -7.603 -11.821 -25.481  1.00 49.40   8       O
ATOM   3677  OG1 TPO F   5      -5.200 -12.467 -25.572  1.00 55.49   8       O
ATOM   3678  CB  TPO F   5      -3.824 -12.505 -25.934  1.00 57.12   6       C
ATOM   3679  CG2 TPO F   5      -3.469 -13.923 -26.369  1.00 57.14   6       C
ATOM   3680  CA  TPO F   5      -2.991 -12.082 -24.729  1.00 57.42   6       C
ATOM   3681  N   TPO F   5      -3.256 -10.658 -24.584  1.00 58.07   7       N
ATOM   3682  C   TPO F   5      -1.523 -12.356 -24.980  1.00 58.27   6       C
ATOM   3683  O   TPO F   5      -0.801 -11.544 -25.752  1.00 57.29   8       O
ATOM   3684  N   PRO F   6      -1.153 -13.293 -24.105  1.00 61.36   7       N
ATOM   3685  CA  PRO F   6       0.332 -13.349 -24.226  1.00 62.33   6       C
ATOM   3686  CB  PRO F   6       0.746 -14.060 -22.935  1.00 62.12   6       C
ATOM   3687  CG  PRO F   6      -0.506 -14.752 -22.491  1.00 62.02   6       C
ATOM   3688  CD  PRO F   6      -1.596 -13.761 -22.781  1.00 61.75   6       C
ATOM   3689  C   PRO F   6       0.781 -14.173 -25.426  1.00 62.85   6       C
ATOM   3690  O   PRO F   6       0.027 -15.003 -25.931  1.00 63.05   8       O
ATOM   3691  N   LEU F   7       2.012 -13.945 -25.866  1.00 63.66   7       N
ATOM   3692  CA  LEU F   7       2.579 -14.672 -26.994  1.00 64.38   6       C
ATOM   3693  CB  LEU F   7       3.904 -14.039 -27.415  1.00 64.68   6       C
ATOM   3694  CG  LEU F   7       4.503 -14.555 -28.720  1.00 65.80   6       C
ATOM   3695  CD1 LEU F   7       3.411 -14.781 -29.760  1.00 66.75   6       C
ATOM   3696  CD2 LEU F   7       5.559 -13.587 -29.234  1.00 66.83   6       C
```

```
ATOM   3697  C   LEU F   7       2.786 -16.146 -26.663  1.00 64.48    6           C
ATOM   3698  O   LEU F   7       2.810 -16.535 -25.493  1.00 64.81    8           O
ATOM   3699  O   WAT W   1      24.634   2.439  -7.629  1.00 30.47    8
ATOM   3700  O   WAT W   2      17.166   2.736   2.573  1.00 32.38    8
ATOM   3701  O   WAT W   3      27.595  14.681  23.241  1.00 29.83    8
ATOM   3702  O   WAT W   4     -27.777  -6.869 -31.502  1.00 32.30    8
ATOM   3703  O   WAT W   5      16.593   0.034   6.219  1.00 30.62    8
ATOM   3704  O   WAT W   6      14.513   2.346   3.344  1.00 31.12    8
ATOM   3705  O   WAT W   7      28.562  12.784  21.663  1.00 34.09    8
ATOM   3706  O   WAT W   8      16.086   2.555   8.816  1.00 29.73    8
ATOM   3707  O   WAT W   9      31.864  20.213   8.572  1.00 28.59    8
ATOM   3708  O   WAT W  10     -30.992 -10.307 -32.072  1.00 29.68    8
ATOM   3709  O   WAT W  11     -26.050   2.758 -38.362  1.00 32.55    8
ATOM   3710  O   WAT W  12      27.489  -8.003   7.433  1.00 33.37    8
ATOM   3711  O   WAT W  13      12.364   0.356   2.037  1.00 28.32    8
ATOM   3712  O   WAT W  14      35.876   2.813  16.912  1.00 37.61    8
ATOM   3713  O   WAT W  15      35.091   0.594  15.613  1.00 33.67    8
ATOM   3714  O   WAT W  16      26.700  14.898  -0.416  1.00 31.60    8
ATOM   3715  O   WAT W  17      33.877 -11.857  -3.921  1.00 36.78    8
ATOM   3716  O   WAT W  18      33.521   9.338  18.361  1.00 36.77    8
ATOM   3717  O   WAT W  19      10.619   5.795   4.943  1.00 38.47    8
ATOM   3718  O   WAT W  20      12.148   9.034   4.695  1.00 33.68    8
ATOM   3719  O   WAT W  21      21.930   2.694  -8.658  1.00 29.23    8
ATOM   3720  O   WAT W  22      28.179  13.091  18.799  1.00 30.76    8
ATOM   3721  O   WAT W  23      24.493  14.521   4.080  1.00 34.88    8
ATOM   3722  O   WAT W  24      19.906   6.992 -13.260  1.00 32.20    8
ATOM   3723  O   WAT W  25       7.557  -8.450   2.900  1.00 63.84    8
ATOM   3724  O   WAT W  26     -27.367   1.228 -36.920  1.00 31.57    8
ATOM   3725  O   WAT W  27      29.654  11.921  -1.388  1.00 29.35    8
ATOM   3726  O   WAT W  28      21.217  -7.633  11.401  1.00 37.46    8
ATOM   3727  O   WAT W  29      25.864  15.796   2.006  1.00 32.25    8
ATOM   3728  O   WAT W  30     -24.053   1.535 -40.872  1.00 33.67    8
ATOM   3729  O   WAT W  31      14.018  13.365 -22.746  1.00 62.02    8
ATOM   3730  O   WAT W  32       9.697   5.905   9.452  1.00 42.01    8
ATOM   3731  O   WAT W  33      18.932   3.541  17.552  1.00 58.68    8
ATOM   3732  O   WAT W  34      25.616  10.543  -1.509  1.00 36.69    8
ATOM   3733  O   WAT W  35     -29.998  -8.101 -30.511  1.00 33.22    8
ATOM   3734  O   WAT W  36      35.706  -8.967 -12.207  1.00 33.88    8
ATOM   3735  O   WAT W  37     -28.712 -14.686 -27.659  1.00 40.16    8
ATOM   3736  O   WAT W  38      27.173  -7.960  -1.547  1.00 30.64    8
ATOM   3737  O   WAT W  39      36.208  10.457  -1.144  1.00 58.59    8
ATOM   3738  O   WAT W  40      25.892  25.852  25.176  1.00 36.79    8
ATOM   3739  O   WAT W  41     -28.690 -12.596 -30.204  1.00 39.60    8
ATOM   3740  O   WAT W  42      11.127   0.674  -6.719  1.00 36.86    8
ATOM   3741  O   WAT W  43      12.634   7.055   6.478  1.00 34.24    8
ATOM   3742  O   WAT W  44      26.064 -12.501 -16.222  1.00 39.99    8
ATOM   3743  O   WAT W  45     -23.089 -17.237 -33.109  1.00 42.46    8
ATOM   3744  O   WAT W  46     -21.850  -0.822 -47.719  1.00 35.95    8
ATOM   3745  O   WAT W  47      33.872  -3.162 -16.877  1.00 34.13    8
ATOM   3746  O   WAT W  48      24.365  34.040  18.694  1.00 41.11    8
ATOM   3747  O   WAT W  49     -28.585   5.275 -23.685  1.00 47.83    8
ATOM   3748  O   WAT W  50      27.720  11.812  -6.004  1.00 52.01    8
ATOM   3749  O   WAT W  51      31.145  11.986  22.378  1.00 36.29    8
ATOM   3750  O   WAT W  52      17.598  21.360  13.347  1.00 40.00    8
ATOM   3751  O   WAT W  53     -27.143 -11.537 -24.167  1.00 42.92    8
ATOM   3752  O   WAT W  54      21.250   3.872  18.777  1.00 62.89    8
ATOM   3753  O   WAT W  55     -11.528   0.411 -15.116  1.00 43.89    8
```

```
ATOM   3754  O   WAT W  56     -32.837  -6.837 -46.162  1.00 40.49    8
ATOM   3755  O   WAT W  57     -13.041   3.240 -39.158  1.00 44.50    8
ATOM   3756  O   WAT W  58      13.747   1.567  -9.380  1.00 38.07    8
ATOM   3757  O   WAT W  59      40.365   3.671  12.390  1.00 40.05    8
ATOM   3758  O   WAT W  60      34.206   3.404  18.995  1.00 35.50    8
ATOM   3759  O   WAT W  61     -25.012  -9.493 -24.326  1.00 33.11    8
ATOM   3760  O   WAT W  62      35.341  25.261  14.453  1.00 37.72    8
ATOM   3761  O   WAT W  63      25.868   5.419  20.464  1.00 42.24    8
ATOM   3762  O   WAT W  64      19.003  16.386  23.952  1.00 42.14    8
ATOM   3763  O   WAT W  65     -37.060  -3.848 -30.377  1.00 41.53    8
ATOM   3764  O   WAT W  66      20.233  10.551 -11.649  1.00 38.76    8
ATOM   3765  O   WAT W  67      36.862  18.521  18.838  1.00 33.85    8
ATOM   3766  O   WAT W  68      -1.871   6.647 -29.708  1.00 46.30    8
ATOM   3767  O   WAT W  69      11.965   3.813   4.062  1.00 40.29    8
ATOM   3768  O   WAT W  70     -27.733  -1.750 -21.747  1.00 39.35    8
ATOM   3769  O   WAT W  71      35.651 -12.751  -6.391  1.00 43.81    8
ATOM   3770  O   WAT W  72      20.746  16.973 -11.203  1.00 69.69    8
ATOM   3771  O   WAT W  73      36.951  -3.167  13.673  1.00 46.35    8
ATOM   3772  O   WAT W  74      33.452  18.001  12.333  1.00 35.28    8
ATOM   3773  O   WAT W  75      39.836  -4.636 -17.377  1.00 41.94    8
ATOM   3774  O   WAT W  76     -26.014  -7.701 -22.795  1.00 36.58    8
ATOM   3775  O   WAT W  77      32.112   6.847  18.892  1.00 35.48    8
ATOM   3776  O   WAT W  78     -24.158 -14.389 -45.202  1.00 49.56    8
ATOM   3777  O   WAT W  79      12.359   7.284   9.231  1.00 35.29    8
ATOM   3778  O   WAT W  80      -7.718 -11.828 -31.638  1.00 43.35    8
ATOM   3779  O   WAT W  81      -4.433  -8.546 -27.834  1.00 46.46    8
ATOM   3780  O   WAT W  82      12.662  11.800   4.986  1.00 37.04    8
ATOM   3781  O   WAT W  83      18.628   4.051 -18.939  1.00 37.36    8
ATOM   3782  O   WAT W  84      41.874  12.668  12.296  1.00 64.40    8
ATOM   3783  O   WAT W  85      24.386  29.260  11.905  1.00 39.20    8
ATOM   3784  O   WAT W  86     -35.916  -9.236 -35.846  1.00 44.00    8
ATOM   3785  O   WAT W  87      24.932  26.384  21.522  1.00 45.38    8
ATOM   3786  O   WAT W  88     -14.850  -1.218 -37.594  1.00 39.23    8
ATOM   3787  O   WAT W  89     -28.949 -10.251 -28.386  1.00 34.73    8
ATOM   3788  O   WAT W  90     -15.971  11.758 -33.481  1.00 66.16    8
ATOM   3789  O   WAT W  91      29.015 -14.521 -18.294  1.00 50.73    8
ATOM   3790  O   WAT W  92     -27.883  -3.366 -24.498  1.00 43.48    8
ATOM   3791  O   WAT W  93      19.046  23.268  24.787  1.00 48.24    8
ATOM   3792  O   WAT W  94      10.369   4.017   7.310  1.00 39.43    8
ATOM   3793  O   WAT W  95      35.601   2.131 -10.164  1.00 44.36    8
ATOM   3794  O   WAT W  96      17.848  24.939  17.240  1.00 43.72    8
ATOM   3795  O   WAT W  97      19.195  -7.795  14.928  1.00 46.11    8
ATOM   3796  O   WAT W  98      41.553   9.708   9.864  1.00 50.38    8
ATOM   3797  O   WAT W  99     -20.658  12.851 -28.172  1.00 48.34    8
ATOM   3798  O   WAT W 100      23.684  14.223  26.217  1.00 56.84    8
ATOM   3799  O   WAT W 101      -9.687 -13.780 -31.210  1.00 41.65    8
ATOM   3800  O   WAT W 102     -12.758   6.302 -35.847  1.00 44.53    8
ATOM   3801  O   WAT W 103      34.728   8.879  -6.401  1.00 54.55    8
ATOM   3802  O   WAT W 104      21.203  -8.624  20.819  1.00 49.17    8
ATOM   3803  O   WAT W 105     -31.852  -0.946 -28.601  1.00 40.67    8
ATOM   3804  O   WAT W 106      -9.893   3.411 -35.561  1.00 45.86    8
ATOM   3805  O   WAT W 107      39.989   1.169  12.158  1.00 41.22    8
ATOM   3806  O   WAT W 108     -29.350  -1.852 -27.097  1.00 41.79    8
ATOM   3807  O   WAT W 109      32.061   6.131  -8.513  1.00 40.19    8
ATOM   3808  O   WAT W 110     -13.807  11.289 -29.680  1.00 59.37    8
ATOM   3809  O   WAT W 111       7.858 -11.260   0.049  1.00 60.48    8
ATOM   3810  O   WAT W 112      16.627   1.411 -12.612  1.00 40.75    8
```

```
ATOM   3811  O   WAT W 113     -19.983  14.426 -30.108  1.00 60.48    8
ATOM   3812  O   WAT W 114      12.245  18.538 -24.003  1.00 82.55    8
ATOM   3813  O   WAT W 115      39.571  15.004  13.995  1.00 37.84    8
ATOM   3814  O   WAT W 116      33.463  -5.785 -20.431  1.00 58.24    8
ATOM   3815  O   WAT W 117     -26.072   7.203 -21.426  1.00 55.49    8
ATOM   3816  O   WAT W 118      18.188  20.859   6.035  1.00 42.20    8
ATOM   3817  O   WAT W 119       5.384  -1.239 -31.530  1.00 59.18    8
ATOM   3818  O   WAT W 120      20.262 -15.072 -13.492  1.00 51.73    8
ATOM   3819  O   WAT W 121      30.189  11.922  24.851  1.00 49.55    8
ATOM   3820  O   WAT W 122      10.788  -2.015  15.189  1.00 59.50    8
ATOM   3821  O   WAT W 123      -7.050  -8.261 -24.625  1.00 45.64    8
ATOM   3822  O   WAT W 124      18.191  23.083  19.249  1.00 41.19    8
ATOM   3823  O   WAT W 125      43.545  -1.639   7.512  1.00 69.29    8
ATOM   3824  O   WAT W 126     -14.472   0.948 -39.333  1.00 46.33    8
ATOM   3825  O   WAT W 127     -31.621  -6.535 -29.094  1.00 39.11    8
ATOM   3826  O   WAT W 128     -35.231  -1.122 -27.823  1.00 49.91    8
ATOM   3827  O   WAT W 129     -19.094  13.991 -27.043  1.00 56.45    8
ATOM   3828  O   WAT W 130      38.995  -6.826 -15.834  1.00 50.99    8
ATOM   3829  O   WAT W 131     -11.364   6.658 -38.443  1.00 49.38    8
ATOM   3830  O   WAT W 132     -10.858  -6.847 -15.161  1.00 45.27    8
ATOM   3831  O   WAT W 133      33.263  13.504  27.027  1.00 49.51    8
ATOM   3832  O   WAT W 134      -9.470   5.931 -46.144  1.00 79.22    8
ATOM   3833  O   WAT W 135      17.824  26.088  20.282  1.00 56.19    8
ATOM   3834  O   WAT W 136      14.973  20.317   5.379  1.00 60.66    8
ATOM   3835  O   WAT W 137       9.146   7.236   3.036  1.00 47.76    8
ATOM   3836  O   WAT W 138      25.903  13.234  -2.501  1.00 41.93    8
ATOM   3837  O   WAT W 139      29.480  14.136 -11.457  1.00 62.93    8
ATOM   3838  O   WAT W 140      40.320   6.951   3.255  1.00 42.71    8
ATOM   3839  O   WAT W 141     -22.104   6.671 -41.503  1.00 54.26    8
ATOM   3840  O   WAT W 142      14.225 -11.428  -3.778  1.00 70.87    8
ATOM   3841  O   WAT W 143      20.799  -7.861  24.532  1.00 63.06    8
ATOM   3842  O   WAT W 144      36.018  -1.336  -5.770  1.00 73.31    8
ATOM   3843  O   WAT W 145      17.809  -8.084  11.994  1.00 63.01    8
ATOM   3844  O   WAT W 146      31.942  -6.401 -22.214  1.00 62.79    8
ATOM   3845  O   WAT W 147     -25.476  -5.436 -21.368  1.00 43.05    8
ATOM   3846  O   WAT W 148      22.760   4.718 -22.884  1.00 54.95    8
ATOM   3847  O   WAT W 149      13.421  -9.857   6.966  1.00 49.49    8
ATOM   3848  O   WAT W 150      13.765   9.827 -16.866  1.00 74.60    8
ATOM   3849  O   WAT W 151     -32.735  -9.192 -28.257  1.00 51.17    8
ATOM   3850  O   WAT W 152      25.500   3.281 -22.705  1.00 53.30    8
ATOM   3851  O   WAT W 153      18.235  -0.257  15.603  1.00 38.90    8
ATOM   3852  O   WAT W 154      -6.061 -14.604 -28.731  1.00 54.75    8
ATOM   3853  O   WAT W 155      40.951   8.546   0.629  1.00 62.07    8
ATOM   3854  O   WAT W 156      32.698  22.571   7.376  1.00 53.61    8
ATOM   3855  O   WAT W 157     -30.708   0.047 -42.456  1.00 58.10    8
ATOM   3856  O   WAT W 158     -19.452 -17.048 -28.371  1.00 49.53    8
ATOM   3857  O   WAT W 159     -34.314  -0.554 -35.382  1.00 48.91    8
ATOM   3858  O   WAT W 160       6.903   3.894 -31.587  1.00 70.11    8
ATOM   3859  O   WAT W 161     -30.049   2.673 -39.111  1.00 52.69    8
ATOM   3860  O   WAT W 162       8.467  12.865 -17.494  1.00 65.54    8
ATOM   3861  O   WAT W 163      33.864  18.806  10.016  1.00 44.82    8
ATOM   3862  O   WAT W 164      10.938  -9.972  -8.363  1.00 54.44    8
ATOM   3863  O   WAT W 165     -20.769  15.416 -25.966  1.00 61.44    8
ATOM   3864  O   WAT W 166      29.110  -7.868   4.834  1.00 59.06    8
ATOM   3865  O   WAT W 167      16.795  17.929  -7.581  1.00 62.10    8
ATOM   3866  O   WAT W 168      -4.711 -12.245 -31.586  1.00 50.04    8
ATOM   3867  O   WAT W 169      19.618  32.961  13.177  1.00 49.55    8
```

```
ATOM   3868  O   WAT W 170      28.062  25.357  22.310  1.00 57.60    8
ATOM   3869  O   WAT W 171       0.867  -2.085   3.050  1.00 65.30    8
ATOM   3870  O   WAT W 172     -21.887  16.627 -31.029  1.00 70.31    8
ATOM   3871  O   WAT W 173      25.330 -14.416 -19.149  1.00 44.85    8
ATOM   3872  O   WAT W 174      13.027  -0.641  -7.690  1.00 40.87    8
ATOM   3873  O   WAT W 175      29.491  15.933  -2.541  1.00 46.06    8
ATOM   3874  O   WAT W 176      28.804   2.041 -24.955  1.00 50.03    8
ATOM   3875  O   WAT W 177     -10.519   3.857 -38.349  1.00 56.56    8
ATOM   3876  O   WAT W 178     -25.503 -15.400 -42.858  1.00 45.25    8
ATOM   3877  O   WAT W 179     -37.796  -1.357 -30.241  1.00 63.19    8
ATOM   3878  O   WAT W 180      -3.515 -12.703 -37.655  1.00 72.93    8
ATOM   3879  O   WAT W 181      21.438  -3.901  24.689  1.00 64.51    8
ATOM   3880  O   WAT W 182     -16.008 -13.906 -23.419  1.00 63.43    8
ATOM   3881  O   WAT W 183      35.740  -8.463  -1.794  1.00 61.46    8
ATOM   3883  O   WAT W 184     -10.500   2.764 -15.695  1.00 70.02    8
ATOM   3884  O   WAT W 185      -9.860 -10.367 -26.412  1.00 47.62    8
ATOM   3885  O   WAT W 186     -38.554  -6.542 -30.237  1.00 62.93    8
ATOM   3886  O   WAT W 187     -32.714  -3.395 -28.295  1.00 48.28    8
ATOM   3887  O   WAT W 188      31.369  12.845   7.044  1.00 56.30    8
ATOM   3888  O   WAT W 189     -13.854 -17.255 -39.219  1.00 77.03    8
ATOM   3889  O   WAT W 190      38.132   5.328   1.621  1.00 53.14    8
ATOM   3890  O   WAT W 191     -29.743 -10.731 -22.814  1.00 56.88    8
ATOM   3891  O   WAT W 192      16.319  -7.567 -19.734  1.00 68.76    8
ATOM   3892  O   WAT W 193      20.905   5.870  21.460  1.00 58.69    8
ATOM   3893  O   WAT W 194      -2.078  -9.343 -26.709  1.00 45.32    8
ATOM   3894  O   WAT W 195     -27.973 -17.217 -34.661  1.00 50.54    8
ATOM   3895  O   WAT W 196       8.090  -6.038 -12.667  1.00 72.72    8
ATOM   3896  O   WAT W 197       5.456  -5.313 -29.937  1.00 64.35    8
ATOM   3897  O   WAT W 198     -17.580  12.084 -27.772  1.00 61.72    8
ATOM   3898  O   WAT W 199      39.310   5.354  15.767  1.00 65.14    8
ATOM   3899  O   WAT W 200      27.072  -1.823  -7.016  1.00 56.78    8
ATOM   3900  O   WAT W 201     -33.783  -9.367 -42.448  1.00 59.22    8
ATOM   3901  O   WAT W 202      32.480   1.951 -21.517  1.00 51.48    8
ATOM   3902  O   WAT W 203      27.509   7.904 -14.495  1.00 62.04    8
ATOM   3903  O   WAT W 204      -6.762 -19.906 -37.936  1.00 67.41    8
ATOM   3904  O   WAT W 205      10.151   2.040  -8.820  1.00 63.45    8
ATOM   3905  O   WAT W 206      42.308  11.982   5.704  1.00 70.95    8
ATOM   3906  O   WAT W 207      32.614   9.993  21.419  1.00 65.65    8
ATOM   3907  O   WAT W 208     -19.924 -12.576 -42.478  1.00 64.81    8
ATOM   3908  O   WAT W 209      -0.031   5.343 -30.924  1.00 61.37    8
ATOM   3909  O   WAT W 210       7.595   4.610  -1.989  1.00 63.64    8
ATOM   3910  O   WAT W 211       9.965   9.231   3.622  1.00 60.62    8
ATOM   3911  O   WAT W 212      23.641  28.537  22.061  1.00 66.92    8
ATOM   3912  O   WAT W 213      -4.088 -10.684 -29.666  1.00 66.92    8
ATOM   3913  O   WAT W 214       4.345  -9.263 -30.965  1.00 66.64    8
ATOM   3914  O   WAT W 215      26.160  29.184  20.917  1.00 71.88    8
ATOM   3915  O   WAT W 216      37.856   8.051  -5.026  1.00 69.47    8
ATOM   3916  O   WAT W 217     -27.466   0.737 -21.368  1.00 61.39    8
ATOM   3918  O   WAT W 218      23.412 -16.483 -20.202  1.00 69.69    8
ATOM   3919  O   WAT W 219      28.293 -13.330  -8.770  1.00 67.32    8
ATOM   3920  O   WAT W 220       3.457  -0.665   5.706  1.00 75.41    8
ATOM   3921  O   WAT W 221      21.431 -19.910 -19.507  1.00 66.40    8
ATOM   3922  O   WAT W 222      35.336   2.654  -5.715  1.00 72.34    8
ATOM   3923  OW0 WAT W 223      35.726  24.518   9.473  1.00 49.00    8
ATOM   3924  OW0 WAT W 224      14.105   8.614  -1.895  1.00 69.00    8
ATOM   3925  OW0 WAT W 225      -5.513  12.590 -37.262  1.00 69.00    8
ATOM   3926  OW0 WAT W 226      28.752  28.494  20.210  1.00 70.00    8
```

ATOM	3927	OW0	WAT	W	227	20.227	15.904	-8.842	1.00	70.00	8
ATOM	3928	OW0	WAT	W	228	7.887	3.313	-4.421	1.00	71.00	8
ATOM	3929	OW0	WAT	W	229	18.680	0.000	18.315	1.00	71.00	8
ATOM	3930	OW0	WAT	W	230	-21.527	-17.229	-35.367	1.00	71.00	8
ATOM	3931	OW0	WAT	W	231	-32.631	-10.602	-30.315	1.00	72.00	8
ATOM	3932	OW0	WAT	W	232	-29.535	-7.952	-51.156	1.00	72.00	8
ATOM	3933	OW0	WAT	W	233	31.358	15.241	30.315	1.00	72.00	8
ATOM	3934	OW0	WAT	W	234	14.620	-11.265	-22.105	1.00	72.00	8
ATOM	3935	OW0	WAT	W	235	-31.286	7.289	-31.578	1.00	72.00	8
ATOM	3936	OW0	WAT	W	236	6.255	7.289	1.895	1.00	72.00	8
ATOM	3937	OW0	WAT	W	237	-3.231	23.193	-21.473	1.00	73.00	8
ATOM	3938	OW0	WAT	W	238	-6.016	-5.301	-28.420	1.00	73.00	8
ATOM	3939	OW0	WAT	W	239	12.255	-4.639	-25.262	1.00	73.00	8
ATOM	3940	OW0	WAT	W	240	8.824	-0.663	12.631	1.00	73.00	8
ATOM	3941	OW0	WAT	W	241	35.953	-5.301	-5.684	1.00	73.00	8
ATOM	3942	OW0	WAT	W	242	-20.326	2.651	-34.104	1.00	73.00	8
ATOM	3943	OW0	WAT	W	243	17.192	23.855	22.105	1.00	73.00	8
ATOM	3944	OW0	WAT	W	244	9.566	-10.602	-22.736	1.00	73.00	8
ATOM	3945	OW0	WAT	W	245	-16.351	-1.325	-14.526	1.00	73.00	8
ATOM	3946	OW0	WAT	W	246	26.578	26.506	2.526	1.00	74.00	8
ATOM	3947	OW0	WAT	W	247	-9.858	8.614	-39.157	1.00	74.00	8

□□□
□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□

Figures were produced with Ribbons (Carson, J. *Appl. Crystallogr.* 24:958-961, 1991) or SPOCK.

Plk1 PBD Binding to Cellular Substrates

HeLa cells were transfected with His/Xpress-tagged Plk1 (residues 326-603 or 326-506) or myc-tagged Plk1 (full-length). They were allowed to recover for 17 hours and then arrested in G2/M by treatment with nocodazole (50 ng/mL) for 14 hours. Cells were lysed in 25 mM Tris/HCl (pH7.5) containing 125 mM NaCl, 0.5% NP-40, 5 mM EDTA, 2 mM DTT, 4 μg/mL pepstatin, 4 μg/mL aprotinin, 4 μg/mL leupeptin, 1 mM Na_3VO_4, 50 mM NaF, and 1 μM microcystin. Lysates were incubated with 5 μL Ni^{2+} beads or 5 μL α-myc-conjugated beads (Santa Cruz Biotechnology) for 90 minutes at 4°C. Beads were washed four times with lysis buffer. Precipitated proteins were eluted in sample buffer and detected by blotting with polyclonal anti-Cdc25C (Santa Cruz Biotechnology). Point mutations of

Plk1 were constructed using the QuickChange site-directed mutagenesis system (Stratagene, La Jolla, CA) and verified by DNA sequencing.

Centrosomal Localization of the Plk1 PBD

U2OS cells were cultured in 8-well chamber slides and arrested in G2/M by treatment with nocodazole (50 ng/mL) for 14 hours. After rinsing with PBS, cells were incubated with 4 μM GST-Plk1 PBD (residues 326-603) and Streptolysin-O (1 U/ml) in permeabilization buffer (25 mM HEPES (pH 7.9), 100 mM KCl, 3 mM NaCl, 200 mM sucrose, 20 mM NaF, 1 mM $NaOVO_4$) for 20 minutes at 37°C. Cells were fixed in 3% paraformaldehyde/2% sucrose for 10 minutes at room temperature and extracted with a 0.5% Triton X-100 solution containing 20 mM Tris-HCl (pH 7.4), 50 mM NaCl, 300 mM sucrose, and 3 mM $MgCl_2$ for 10 minutes at Room temperature. Slides were stained with Alexa Fluor 488-conjugated anti-GST (Molecular Probes, Eugene, OR) and monoclonal anti-γ-tubulin (Sigma) antibodies at 4°C overnight, then stained with a Texas Red conjugated anti-mouse secondary antibody for 60 minutes at room temperature and counterstained with 4 μg/ml DAPI. Cells were examined using a Nikon Eclipse E600 fluorescence microscope equipped with a SPOT RT camera and software (Diagnostic Instruments Livingston, Scotland). Images were analyzed using NIH Image.

Cell Cycle Analysis

HeLa cells were transfected with wild-type and mutant forms of GFP-tagged Plk1 (residues 326-603) for 32 hours. Media containing floating cells was retained, and attached cells were released from plates by trypsinization. The two cell populations were combined, washed with PBS, and stained with Hoechst 33342 (10μg/mL) for 30 minutes at 37°C in DMEM/10%FBS ($1x10^6$ cells/mL). Dead cells were stained by incubation with propridium iodide (5 μg/mL) for 5 minutes at 4°C. GFP, Hoechst 33342, and propidium iodide fluorescent signals

were quantitated on a FAC Star Plus (Becton Dickinson, Franklin Lakes, N.J) cell sorting machine using Cell Quest software. Cell cycle analysis of the total live cell population (no propidium iodide staining) and live GFP-expressing cells (no propidium staining and GFP positive) was performed using Modfit 2.0.

Plk1 Kinase Assays

SF9 cells infected with baculoviral GST-Plk1 (full-length) were lysed in 20 mM Hepes/KOH (pH 7.5), 135 mM NaCl, 1% NP40, 5 mM EGTA, 5μM β-mercaptoethanol, 35 mM NaF, 0.5 mM Na_3VO_4, 20 mM β-glycerolphosphate, 3 μM microcystin, 1 μM okadaic acid, 10 μg/mL pepstatin, 10 μg/mL leupeptin, and 10 μg/mL aprotinin. Lysates were incubated for 2 hours at 4°C with glutathione beads, which were subsequently washed five times with 20 mM Hepes/KOH (pH 7.5), 415 mM NaCl, 0.1% CHAPS, 5 mM EGTA, 5μM β-mercaptoethanol, 35 mM NaF, and 0.5 mM Na_3VO_4 at 4°C. Bound proteins were eluted with a buffer containing 30 mM glutathione, 50 mM Hepes/KOH (pH 8.0), 25mM NaCl, 2mM $MgCl_2$, 1mM EGTA, and 5μM β-mercaptoethanol and dialyzed against 10mM Hepes, 10mM NaCl, 1mM EGTA, 1mM DTT for 3 hours at 4°C. Kinase reactions were performed in 20 mM Hepes/KOH (pH7.5), 15 mM KCl, 10 mM $MgCl_2$, 1 mM EGTA, 100 μM ATP, 5μCi γ-[^{32}P]-ATP, 1 mM DTT, and 0.1 μg/μgL casein for 15 minutes at 30°C. Reaction aliquots were removed at various time points, added to sample buffer, and boiled to arrest phosphorylation. ^{32}P-incorporation into casein was determined by SDS-PAGE electrophoresis, autoradiography, and densitometry using ImageQuant software (Molecular Dynamics). For peptide activation experiments, 250 μM of the PBD optimal phosphopeptide (MAGPMQSpTPLNGAKK) (SEQ ID NO: 3) or its non-phosphorylated counterpart (MAGPMQSTPLNGAKK) (SEQ ID NO: 34) were pre-incubated with GST-Plk1 for 5 minutes at room temperature.

Molecular Modeling *in silico*

The present invention provides an exemplary crystallized PBD-phosphopeptide complex and the atomic structural coordinates of this complex. The key structural features of the complex, particularly the shape of the substrate binding site, are useful in methods for designing or identifying selective inhibitors of a Polo-like kinase polypeptide, such as Plk-1, and in solving the structures of other proteins with similar features. The structure coordinates of this complex are encoded in a data storage medium, submitted herewith, for use with a computer for graphical three-dimensional representation of the structure and for computer-aided molecular design of new inhibitors. The differences in three-dimensional structure between PLK-1 and related proteins with known structures can be used to optimize selectivity of an inhibitor for PBD. In addition to the structural differences described herein, other differences between Plk-1 and other proteins can also be identified by a skilled artisan.

The three-dimensional atomic structures reported herein can be readily used as a template for selecting potent inhibitors, such as small molecules or peptidomimetics that are designed to "fit" into the binding interface. Methods for designing peptidomimetics using rational drug design are known to the skilled artisan, and are described, for example, in U.S. Patent Nos: 6,225,076; 6,171,804; and in Han *et al.* (*Bioorg Med Chem. Lett*, 10:39-43, 2000). Peptidomimetics capable of inhibiting complex formation can be identified, for example, through the use of computer modeling using a docking program such as GRAM, DOCK, or AUTODOCK (Dunbrack et al., *Folding & Design*, 2:27-42, 1997). This procedure can include computer fitting of candidate compounds to a the binding interface of a particular polypeptide to determine whether the shape and chemical structure of the potential ligand will allow it to bind within the structure of the polypeptide. Many methods can be used for this purpose such as, but not limited to, fast shape matching (Dock [Kuntz et al., *J. Mol. Biol.*, 161:269-288, 1982]; Eudock [Perola et al., *J. Med. Chem.*, 43:401-408, 2000]), incremental

construction (FlexX [Rarey et al., *J Mol Biol*, 261, 470-89, 1996]; HAMMERHEAD [Welch et al., *Chem. Biol.*, 3, 449-462, 1996]), TABU search (Pro_Leads [Baxter et al., *Proteins* 33:367-382, 1998]; SFDock [Hou et al., *Protein Eng.* 12:639-647, 1999]), genetic algorithms (GOLD [Gold et al., *J. Mol. Biol.* 267:727-748, 1997]; AutoDock 3.0 [Morris et al., *J. Comput. Chem.*, 19:1639-1662, 1998]; Gambler [Charifson et al., *J. Med. Chem.*, 42:5100-5109, 1999]), evolutionary programming [Gehlhaar et al., *Chem. Biol.*, 2:317-324, 1995], simulated annealing (AutoDock 2.4 [Goodsell et al., *Proteins*, 8:195-202, 1990]), Monte Carlo simulations (MCDock [Liu et al., *J. Comput.-Aided Mol. Des.*, 13:435-451, 1999]; QXP [McMartin et al., *J. Comput.-Aided Mol. Des.*, 11:333-344, 1997]), and distance geometry (Dockit [Metaphorics LLC, Piemont, CA 94611 www.metaphorics.com]).

Those skilled in the art can readily identify many small molecules or fragments as hits. If desired, one can link the different functional groups or small molecules identified by the above procedure into a single, larger molecule. The resulting molecule is likely to be more potent and have higher specificity. The affinity and/or specificity of a hit can also be improved by adding more atoms or fragments that will interact with the target protein. The originally defined target site can be readily expanded to allow further necessary extension. Selected compounds may be systematically modified by computer modeling programs to identify peptidomimetics having the greatest therapeutic potential. Alternatively, candidate compounds are selected from chemical libraries, or are synthesized *de novo*.

The structural analysis disclosed herein in conjunction with computer modeling allows the selection of a finite number of rational chemical modifications. Thus, using the complex structure disclosed herein and computer modeling, a large number of candidate compounds can be rapidly screened *in silico*, and the most promising candidates can be identified. Candidate compounds, such as peptidomimetics, are then verified *in vitro* or *in vivo*, for

example, by determining the effect of the candidate compound on PBD/phosphopeptide binding, Polo-like kinase biological activity, cell cycle regulation, apoptosis, or cell proliferation.

pSer/pThr-binding domains function in the cellular response to genotoxic stress

Signal transduction by protein kinases in eukaryotes results in the directed assembly of multi-protein complexes at specific locations within the cell (Pawson et al., *Science* 300:445-52, 2003). This process is particularly evident following DNA damage, where activation of DNA damage kinases results in the formation of protein-protein complexes at discrete foci within the nucleus (Zhou et al., *Nature* 408:433-9, 2000).

In many cases, kinases directly control the formation of these multi-protein complexes by generating specific phosphorylated-motif sequences; modular binding domains then recognize these short phospho-motifs to mediate protein-protein interactions. The first phosphopeptide-binding modules that were recognized, SH2 and PTB domains, bind specificially to pTyr-containing sequences (Pawson et al., *Science* 278:2075-80, 1997; Kuriyan et al., *Annu Rev Biophys Biomol Struct* 26:259-88, 1997;Yaffe, *Nat Rev Mol Cell Biol* 3:177-86, 2002). As detailed above, a number of modular domains that specifically recognize short pSer/pThr-containing sequences have now been identified, including 14-3-3 proteins, WW domains, FHA domains, and the C-terminal domain of Polo-like kinases (Yaffe et al., *Structure* 9:R33-8, 2001; Yaffe et al., *Curr Opin Cell Biol* 13:131-8, 2001; Elia et al., *Science* 299:1228-31, 2003). All of these pSer/pThr-binding domains participate in cell cycle regulation and the cellular response to genotoxic stress.

The PTIP tandem C-terminal BRCT pair is necessary and sufficient for phospho-specific binding

Using the proteomic screening approach (Elia et al., *Science* 299:1228-31, 2003). described herein, we have now identified novel modular pSer/pThr-binding domains involved in the DNA damage response. Following γ-irradiation, phosphoinositide-like kinases including ATM/ATR and DNA-PK phosphorylate transcription factors, DNA repair proteins, protein kinases and scaffolds on Ser-Gln and Thr-Gln motifs (Abraham, *Genes Dev* 15:2177-96, 2001). We therefore constructed an oriented peptide library biased to resemble the (pSer or pThr)-Gln motif generated by ATM and ATR (Kim et al., *J Biol Chem* 274:37538-43, 1999; O'Neill et al., *J Biol Chem* 275:22719-27, 2000). (Figure 17A legend). An immobilized form of this library was used in an interaction screen against a library of proteins produced by *in vitro* expression cloning (Lustig et al., *Methods Enzymol* 283:83-99, 1997). The amino acids Arg, Lys, and His were intentionally omitted from the degenerate positions in the peptide library to decrease the likelihood of identifying phosphopeptide-binding domains such as 14-3-3, which target basophilic motifs generated by kinases such as AKT, PKA, and PKCs. To control for phosphorylation-independent binding, an identical peptide library was constructed with (Ser or Thr)-Gln substituted for (pSer or pThr)-Gln.

The phosphorylated and non-phosphorylated peptide libraries were immobilized on streptavidin beads, and screened against approximately 96,000 *in vitro* translated (IVT) polypeptides (960 pools each encoding ~ 100 transcripts) over a 10 week period using a high-throughput approach. The majority of IVT products either failed to bind to either of the immobilized peptide libraries or bound slightly better to the non-phosphorylated control (Figure 17A). Several pools were found to contain cDNAs encoding proteins which bound preferentially to the (pSer or pThr)-Gln library. Pool EE11 contained the strongest phosphopeptide-binding clone, EE11-9, which when sib-selected, was found to encode the C-terminal 70% of the human Pax2 *trans*-activation domain-interacting

protein (PTIP) (Figure 17B) (Lechner et al., *Nucleic Acids Res* 28:2741-51, 2000; Cho et al., *Mol Cell Biol* 23:1666-73, 2003). Originally identified in a yeast 2-hybrid screen using Pax2 as bait (Lechner et al., *Nucleic Acids Res* 28:2741-51, 2000), PTIP appears to play a critical role in the DNA damage response pathway (Cho et al., *Mol Cell Biol* 23:1666-73, 2003), as well as in facilitating transcriptional responses downstream of TGF-β-Smad2 signaling (Shimizu et al., *Mol Cell Biol* 21:3901-12, 2001).

Full-length PTIP transcripts also displayed preferential binding to (pSer or pThr)-Gln peptides, though the differential binding was somewhat less pronounced, suggesting that the C-terminal fragment of PTIP likely contains a discrete phosphopeptide binding module. In addition to its Gln-rich region, human PTIP contains 4 BRCT domains, which are known protein-protein interaction modules present in many DNA damage response and cell cycle checkpoint proteins z (Huyton et al., *Mutat Res* 460:319-32, 2000). A series of deletion constructs was therefore generated and analyzed for phosphopeptide-specific binding (Figure 17B). A construct containing only the tandem 3rd and 4th BRCT domains showed strong and specific binding to the (pSer or pThr)-Gln library. Constructs of PTIP lacking both of these domains failed to bind or lacked phospho-discrimination. Furthermore, neither the 3rd or 4th BRCT domains alone bound to phosphopeptides, suggesting that the PTIP tandem C-terminal BRCT pair functions as a single module that is necessary and sufficient for phospho-specific binding.

Tandem BRCT domains function as single unit to mediate phosphopeptide-binding

BRCT domains are often found in tandem pairs, or multiple copies of tandem pairs. To investigate whether (pSer- or pThr)-binding is a general feature of these domains, we screened tandem BRCT pairs from a number of other DNA

damage proteins (Figure 18A). Like PTIP, the BRCA1 C-terminal BRCT domains also showed phospho-specific binding. Neither of the BRCA1 BRCT domains alone was sufficient for phospho-specific interactions, again suggesting that the tandem BRCT domains are functioning as a single unit. This observation is in excellent agreement with limited proteolysis and X-ray crystallography studies in which the tandem BRCA1 BRCT domains together with the inter-domain linker behave as a single stable fragment (Williams et al., *Nat Struct Biol* 8:838-42, 2001). In contrast to PTIP and BRCA1, phospho-specific binding to the tandem BRCT domains of MDC1 or 53BP1 was not observed, and only a very low amount of phospho-specific binding for Rad9 was detected, suggesting that the phosphopeptide-binding function is present in only a subset of tandem BRCT domains.

Identification of Optimal tandem BRCT domain-binding peptide

Modular domains identified by binding to bead-immobilized phosphopeptide libraries are directly amenable to determination of their optimal binding motif by traditional peptide library screening (Yaffe et al., *Methods Enzymol* 328:157-70, 2000; Elia et al., *Science* 299:1228-31, 2003). We determined the optimal pSer/pThr binding motifs for the tandem C-terminal BRCTs in PTIP and BRCA1 using (pSer or pThr)-Gln, pSer- and pThr-containing peptide libraries (Figure 18B and 18C, Table 46). For the PTIP C-terminal BRCT, the binding motifs using (pSer or pThr)-Gln, pThr-, and two pSer-containing peptide libraries were identified as SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 112, and SEQ ID NO: 113, respectively (shown in Table 6). For the BRCA1 C-terminal BRCT, the binding motifs using (pSer or pThr)-Gln, pThr-, and two pSer-containing peptide libraries were identified as SEQ ID NO: 114, SEQ ID NO: 115, SEQ ID NO: 116, and SEQ ID NO: 117, respectively (shown in Table 6).

Table 6 Phosphoserine and phosphothreonine peptide motif selection by PTIP and BRCA1 Tandem BRCT motifs

Phosphoserine and Phosphothreonine Peptide

Motif Selection by PTIP and BRCA1 Tandem BRCT Domains

PTIP

-4	-3	-2	-1		+1	+2	+3	+4	+5
X	Y (1.5)	G (2.3) D (1.5) E (1.4)	L (2.6) I (2.5) M (2.5) V (1.9)	**pS/pT**	Q	V (3.8) I (2.8)	F (7.0) L (4.3) I (4.1)	P (1.6)	I (2.9) F (2.7) L (2.4) V (2.0) Y (2.0)
X	X	E (1.3)	I (1.4) M (1.4) V (1.4) L (1.3)	**PS**	F (1.7) I (1.5) Q (1.5) Y (1.3)	V (1.8) T (1.5)	F	X	I (1.9) F (1.7) M (1.6) L (1.4)
G (1.6)	Y (1.1)	D (1.2) E (1.1)	L (1.2) I (1.2) M (1.2)	**PS**	Q (1.3) I (1.3) P (1.2)	V (2.1) I (1.7)	F (2.3) I (2.3) V (1.8) L (1.7) Y (1.5)	P (1.2)	Y (1.3)
X	X	X	I (2.1) L (1.8) W (1.3)	**PT**	Q (1.5) F (1.4) I (1.3)	Y (1.4)	I (1.4) L (1.3) V (1.2)	F (1.5) Y (1.4) P (1.3)	**A**

5

BRCA1									
-4	-3	-2	-1		+1	+2	+3	+4	+5
X	F (1.7) Y (1.6)	D (1.2) E (1.1)	I (1.4) V (1.3) L (1.2) M (1.2)	**pS/pT**	Q	V (3.1) T (2.6) I (2.2) S (1.7)	F (7.5) Y (5.2)	V (1.5) P (1.4)	F (4.5) G (1.8)
X	R (1.5) Y (1.4)	E (1.3) D (1.2)	V (1.4) I (1.3) M (1.3)	**pS**	F (2.1) Y (1.6) I (1.4) Q (1.4)	T (1.9) V (1.7)	F	X	F (1.6) M (1.4) Y (1.3)
X	X	Y (1.2)	X	**pS**	Q (1.4) F (1.3)	V (1.2) I (1.2)	F (2.4) Y (1.5)	I (1.2)	X
X	E (1.5)	D (1.9) E (1.5)	I (1.6) L (1.4)	**pT**	Q (1.5) E (1.4) F (1.3)	D (1.5) Y (1.3) I (1.2)	F (1.9) Y (1.2)	D (1.4) P (1.2)	A

A GST fusion of the PTIP or BRCA1 tandem BRCT domains was screened for binding to four phosphopeptide libraries, which contained the sequences GAXXXB(pS/pT)QJXXXAKKK (SEQ ID NO: 62), GAXXXXpSXXFXXAYKKK (SEQ ID NO: 59), MAXXXXpTXXXXAKKK (SEQ ID NO: 47), and MAXXXXpSpXXXXXAKKK (SEQ ID NO: 58), where X indicates all amino acids except Cys. In the library MAXXXB(pS/pT)QJXXXAKKK (SEQ ID NO: 62) B indicates A, I, L, M, N, P, S, T, V, and J represents a biased mixture of 25% E, 75% X, while X indicates all amino acids except Arg, Cys, His, Lys for all positions in this library. Residues showing strong enrichment are underlined.

Table 6 shows the results of a phosphoserine and phosphothreonine motif selection by PTIP and BRCA1 tandem BRCT domains. A GST fusion of the PTIP or BRCA1 tandem BRCT domains was screened for binding to three phosphopeptide libraries, which contained the sequences MAXXXB(pS/pT)QJXXXAKKK SEQ ID NO:~~53~~ 57, MAXXXXpTXXXXAKKK SEQ ID NO:~~54~~ 47, and MAXXXXSpXXXXXAKKK SEQ ID NO:~~55~~ 58; where X indicates all amino acids except Cys. In the libraries MAXXXB(pS/pT)QJXXXAKKK (SEQ ID NO:~~56~~ 57) and GAXXXXpSXXFXXAYKKK (SEQ ID NO: 59), B indicates A, I,

L, M, N, P, S, T, V; and J represents a biased mixture of 25% E, 75% X, while X indicates all amino acids except Arg, Cys, His, Lys. Residues showing very strong enrichment (ratio >3) are underlined.

PTIP and BRCA1 BRCTs displayed similar, but not identical motifs, with extremely strong selection for aromatic/aliphatic residues, and aromatic residues, respectively, in the (pSer or pThr)+3 position when screened with a (pSer or pThr)-Gln library. Prominent amino acid selection was also observed in the (pSer or pThr)+2 and +5 positions, in addition to more moderate selection at other positions. Because the BRCT domains were isolated in a screen for domains that bind to (pSer or pThr)-Gln motifs, we investigated the relative importance of Gln in the (pSer or pThr)+1 position using individual pThr- or pSer-oriented peptide libraries. This analysis revealed modest selection for Gln in the degenerate +1 position. Furthermore, the absence of a fixed Gln in the +1 position reduced the selection for aromatic and aliphatic residues in the +3 and +5 positions, suggesting that while Gln in the (pSer or pThr)+1 position was not essential, it was clearly a favored residue. In agreement with this finding, we observed considerably stronger binding of the tandem BRCT domains to bead-immobilized (pSer or pThr)-Gln libraries than to libraries containing only a fixed pSer motif (Figure 18A).

On the basis of peptide library data, we defined an optimal tandem BRCT domain-binding peptide as Y-D-I-(pSer or pThr)-Q-V-F-P-F (SEQ ID NO: 60). Isothermal titration calorimetry (ITC) showed that the optimal phosphoserine-containing peptide bound to the tandem C-terminal BRCTs of PTIP with a dissociation constant of 280 nM, and to the BRCT domains of BRCA1 with a dissociation constant of 400 nM (Table 7). Binding affinity results for GAAYDI-pS-QVFPFAKKK (SEQ ID NO: 123), GAAYDI-pT-QVFPFAKKK (SEQ ID NO: 124), GAAYDI-S-QVFPFAKKK (SEQ ID NO: 125), GAAYDI-T-QVFPFAKKK (SEQ ID NO: 126), GAAYDI-pS-QVFPFAKKK (SEQ ID NO: 127), GAAYDI-

S-QVFPFAKKK (SEQ ID NO: 128), and GAAYDI-T-QVFPFAKKK (SEQ ID NO: 129) are shown in Table 7.

Table 7 Peptide binding affinities for the tandem BRCT domains

Table S2. Peptide Binding Affinities for the Tandem BRCT Domains

Peptide	Sequence	$(BRCT)_2$ Domain	K_d
BRCTtide-7pS	GAAYDI-*p*S-QVFPFAKKK	PTIP	280 nM
BRCTtide-7pT	GAAYDI-*p*T-QVFPFAKKK	PTIP	14.3 μM
BRCTtide-7S	GAAYDI- S-QVFPFAKKK	PTIP	N.D.B.
BRCTtide-7T	GAAYDI- T-QVFPFAKKK	PTIP	N.D.B.
BRCTtide-7pS	GAAYDI-*p*S-QVFPFAKKK	BRCA1	400 nm
BRCTtide-7S	GAAYDI- S-QVFPFAKKK	BRCA1	N.D.B.
BRCTtide-7T	GAAYDI- T-QVFPFAKKK	BRCA1	N.D.B.

Isothermal titration calorimetry (ITC) was used to determine binding constants (K_d). All observed binding stoichiometries were consistent with a 1:1 complex of protein and phosphopeptide. N.D.B indicates no detectable binding by ITC for a tandem BRCT domain with a concentration of at least 150μM. pS and pT denote phosphoserine and phosphothreonine, respectively.

PTIP and BRCA1 tandem BRCT domains were purified as GST-fusion proteins from *E. coli* and binding to individual peptides measured by isothermal titration calorimetry. Binding stoichiometries were consistent with a 1:1 complex of protein and phosphopeptide. Replacement of pThr for pSer reduced the affinity of the peptide for the PTIP BRCT domains, while substitution of Thr for pThr abrogated binding altogether.

To further verify motif selection, binding of the tandem BRCT domains to a solid-phase array of immobilized phosphopeptides was performed in which each amino acid flanking the pThr-Gln core (Figure 18D and 18E) or flanking the pSer (Figures 18F and 18G) in the optimal BRCTtide was varied. The resulting selectivities were generally consistent with the results obtained using oriented peptide libraries in solution. Substitution of pSer for pThr significantly enhanced binding for both PTIP and BRCA1, consistent with the ITC results for PTIP.

Substitution of pTyr for pThr eliminated binding altogether, verifying that tandem BRCT domains are pSer/pThr-specific binding modules. As expected, replacement of pThr with Thr, Ser or Tyr abrogated tandem BRCT domain binding.

Tandem BRCT domain binding eliminated by pre-incubation with (pSer or pThr)-Gln peptide library

To examine the role of tandem BRCT domains in binding to ATM/ATR/ATX-phosphorylated proteins after DNA damage, U2OS cell lysates, prior to and following 10 Gy of γ-irradiation, were incubated with GST-$(BRCT)_2$ fusion proteins and blotted with an anti-(pSer or pThr)-Gln motif antibody raised against the phosphorylation motif generated by ATM and ATR (Cell Signaling Technologies) (Figures 19A-19D). Following γ-irradiation, both PTIP and BRCA1 tandem C-terminal BRCTs bound to numerous proteins recognized by the anti-ATM/ATR phosphoepitope motif antibody (Figure 19A). This interaction could be inhibited by pre-incubating the tandem BRCT domains with a (pSer or pThr)-Gln peptide library, but not with a pThr-Pro library or with the non-phosphorylated (Ser or Thr)-Gln library. A time course analysis revealed optimal binding of both the PTIP and BRCA1 BRCT domains to (pSer or pThr)-Gln-containing proteins in irradiated cell lysates at 0.5 and 2 hours after DNA damage (Figure 19B and 19D). Binding was largely eliminated by the optimal BRCTtide (opt), but not by its non-phosphorylated analogue (7T), or by pre-treatment of the cells with caffeine to inhibit ATM and ATR prior to γ-irradiation. In both cases where the phospho-specific interaction was eliminated, we observed a ~170 kDa immunoreactive band in the PTIP BRCT domain pulldowns, but not in the BRCA1 pulldowns; this band likely resulted from an interaction with the PTIP BRCT domains at a site distinct from its phosphopeptide-binding pocket.

Tandem C-terminal BRCT domains are necessary and sufficient for nuclear foci formation following DNA damage

In response to γ-irradiation, the DNA damage protein 53BP1 undergoes phosphorylation by ATM and facilitates the ability of ATM to phosphorylate additional cellular substrates (Schultz et al., *J Cell Biol* 151:1381,2000; Rappold et al., *J Cell Biol* 153:613-20, 2001; Anderson et al., *Mol Cell Biol* 21:1719-29, 2001; Abraham, *Nat Cell Biol* 4:E277-9, 2002; Wang et al., *Science* 298:1435-8, 2002; Fernandez-Capetillo et al., *Nat Cell Biol* 4:993-7, 2002; DiTullio, Jr. et al., *Nat Cell Biol* 4:998-1002, 2002). 53BP1 migrates at a similar Mr as one or more of the bands in Figure 19A and 19B and contains multiple potential Ser/Thr-Gln ATM/ATR phosphorylation sites that closely match the optimal PTIP tandem BRCT-binding motif. Endogenous 53BP1 from U2OS cell lysates bound to the tandem C-terminal BRCT domains of PTIP only following DNA damage (Figure 19C). Similar to the results obtained with the (pSer or pThr)-Gln motif antibody, a time course of cells transfected with HA-tagged 53BP1 revealed optimal binding at 0.5 and 2 hours following γ-irradiation. This binding was inhibited by preincubation with optimal BRCTtide, but was not eliminated by pre-incubation with its non-phosphorylated counterpart. Binding was also eliminated by pre-incubation of the tandem BRCT domains with the (pSer or pThr)-Gln peptide library, but not by pre-incubation with a pThr-Pro library or the non-phosphorylated (Ser or Thr)-Gln library, as well as by treatment with caffeine prior to γ-irradiation or treatment of the lysates with λ-phosphatase following irradiation.

Although PTIP was originally identified as a transcriptional control protein, recent data suggests that PTIP might also be involved in DNA damage signaling (Cho et al., *Mol Cell Biol* 23:1666-73, 2003). Mice homozygous for a PTIP null allele undergo embryonic lethality at E9.5, with evidence of extensive DNA damage and the presence of free DNA ends. Neither fibroblasts nor embryonic stem cells from PTIP null mice could be propagated in culture, and trophoblast

cells, which showed decreased viability in general, showed an increased sensitivity to low doses of ionizing radiation (Cho et al., *Mol Cell Biol* 23:1666-73, 2003). This data, together with our finding that the tandem BRCT domains at the C-terminus of PTIP bind to ATM/ATR phosphorylated proteins, suggested that full-length PTIP might localize at sites of DNA damage *in vivo*.

To investigate this, U2OS cells were transfected with GFP fusions of full-length PTIP, PTIP lacking the last two C-terminal BRCT domains, or the isolated tandem C-terminal BRCT domains alone (Figures 20A-20C). In the absence of irradiation, PTIP was diffusely nuclear with a small amount of cytosolic staining. Two hours following DNA damage, PTIP re-localized into discrete nuclear foci that significantly co-localized with ATM/ATR phosphoepitopes, 53BP1 and phospho-H2AX (Figure 20A). Deletion of the C-terminal BRCTs from PTIP resulted in its constitutive diffuse nuclear and cytoplasmic localization and an inability to form foci after DNA damage (Figure 18B). The isolated PTIP C-terminal tandem BRCT domains, while predominantly diffusely nuclear in the absence of DNA damage, efficiently re-localized into the same punctate nuclear foci after γ-irradiation as full-length PTIP (Figure 18C). Thus, the tandem C-terminal BRCT domains of PTIP, which are necessary and sufficient for binding to (pSer or pThr)-Gln peptides in solution, are necessary and sufficient for nuclear foci formation by full-length PTIP following DNA damage.

Caffeine attenuates recruitment of PTIP to DNA damage foci in response to ionizing radiation (Figures 21A and 21B). U20S cells transfected with full-length PTIP-GFP cDNA were mock treated or pretreated with 10mM caffeine for 70 minutes before exposure to 10Gy ionizing radiation. In reponse to IR ionizing radiation, mock-treated U20S cells formed nuclear foci containing PTIP (in green) and H2AXp (in red); these two proteins co-localize at sites of DNA damage (merge). In response to IR, caffeine treated U20S cells formed reduced numbers of nuclear foci; PTIP was mislocalized and did not form discrete nuclear foci (in green) and there were reduced numbers of H2AXp (in red) containing foci. These

results demonstrate that pretreatment with caffeine effectively abolished co-localization of PTIP and H2AXp (merge).

Our identification of tandem BRCT domains as a new pSer/pThr-binding module targeting ATM and ATR phosphorylation motifs expands the range of functions subserved by this domain in response to DNA damage signaling. Only tandem pairs were observed to function in this capacity, and only a subset of BRCT domains, including those in PTIP and BRCA1, appear to show phospho-specific binding. The important role for tandem BRCT domains as phospho-binding modules is emphasized by the finding that ~80% of germline mutations in BRCA1 result in C-terminal truncations involving the BRCT region, predisposing women to breast and ovarian cancer (Huyton et al., *Mutat Res* 460:319-32, 2000). Interestingly, a BRCA1 cancer-associated mutation in the (BRCT)2 module that ablates critical BRCA1 protein interactions, Met17753Arg (M1775R), fails to bind phosphopeptides (Fig. 2A), even though the M1775R crystal structure is nearly identical to that of the wild-type (BRCT)2 . The finding that BRCT domains bind to pSer-containing peptides more strongly than to pThr-containing peptides is novel since WW domains, 14-3-3 proteins, FHA domains and Polobox domains either bind pThr-peptides better than pSer peptides, or do not bind to pSer-peptides at all (Verdecia et al., *Nat Struct Biol* **7**:639-43, 2000; Durocher *et al.*, *Mol Cell,* 6:1169-1182, 2000; Elia et al., *Science* 299:1228-31, 2003). Intriguingly, ATM and ATR preferentially phosphorylate Ser-Gln over Thr-Gln motifs (Kim et al., *J Biol Chem* 274:37538-43, 1999), suggesting functional convergence between the motifs generated by phosphoinositide-like kinases and the motifs recognized by BRCT domains. The observed BRCT domain selection for aromatic and aliphatic residues in the (pSer or pThr)+3 and +5 positions within their bound substrates exceeds their modest selection for Gln in the +1 position. Thus, only a subset of ATM/ATR phosphorylated substrates are likely to bind with high affinity. Kinases other than Gln-directed kinases might also generate potential BRCT domain-binding motifs. In addition, the results of our screen

provide a molecular rationale for the early embryonic lethality of PTIP knock-out mice with extensive unrepaired DNA ends. The finding that the C-terminal tandem BRCT domains of PTIP bind to ATM/ATR-phosphorylated motifs and localize full-length PTIP to sites of DNA damage strongly suggests that PTIP functions as a key component of the DNA damage response. Interference with the normal process of DNA damage signaling is responsible not only for tumorigenesis but also for tumor cell death in the face of massive DNA damage induced by chemotherapeutic agents, depending on the remaining genetic background of the cancer cell (Scully et al., *Nature* 408:429-32, 2000). Agents that interfere with DNA damage signaling sensitize tumor cells to killing by radiation and chemotherapy. Thus, the phosphopeptide-binding pocket of tandem BRCT domains constitutes a promising target for anti-cancer drug development. \

ATM/ATR/ATX phospho-motif screen for phosphoserine/threonine binding domains

An oriented (pSer/pThr) phosphopeptide library biased toward the phosphorylation motifs for ATM/ATR kinases and its non-phosphorylated counterpart were constructed as follows:

biotin-Z-G-Z-G-G-A-X-X-X-B-(pS/pT)-QJ-X-X-X-A-K-K-K SEQ ID NO:~~57~~ 35 and

biotin-Z-G-Z-G-G-A-X-X-X-B-(S/T)-Q-J-X-X-X-A-K-K-K SEQ ID NO:~~58~~ 61,

where pS denotes phosphoserine; pT phosphothreonine; Z indicates aminohexanoic acid; B represents a biased mixture of the amino acids A, I, L, M, N, P, S, T, V; and J represents a biased mixture of 25% E and 75% X, where "X" denotes all amino acids except Arg, Cys, His, Lys. Streptavidin beads (Pierce, 75pmol/μL gel) were incubated with a ten-fold molar excess of each biotinylated library in 50 mM Tris/HCl (pH7.6), 150 mM NaCl, 0.5% NP-40, 1 mM EDTA, 2 mM DTT and washed five times with the same buffer to remove unbound peptide. The bead-immobilized libraries (10μL of gel) were added to 10 μL of an *in vitro*

translated [^{35}S]-labeled protein pool in 150 µL binding buffer (50 mM Tris/HCl (pH7.6), 150 mM NaCl, 0.5% NP-40, 1 mM EDTA, 2 mM DTT, 8 µg/mL pepstatin, 8 µg/mL aprotinin, 8 µg/mL leupeptin, 800 µM Na3VO4, 25 mM NaF). Each pool consisted of ~100 radiolabeled proteins produced by the PROTEOLINK *in vitro* expression cloning system (Promega, Madison, WI). After incubation at 4°C for 3 hours, the beads were rapidly washed three times 200 µL with binding buffer prior to SDS-PAGE (12.5%) and autoradiography. Positively scoring hits were identified as protein bands that interacted more strongly with the phosphorylated immobilized library than with the unphosphorylated counterpart. Pools containing positively scoring clones were progressively subdivided and re-screened for phosphobinding until single clones were isolated and identified by DNA sequencing.

Cloning, expression, and purification of PTIP and BRCA1

For deletion mapping of the PTIP and BRCA1 BRCT phospho-binding region and for expression of MDC1, 53BP1 and Rad9 (Figure 17-18), fragments were generated by PCR for *in vitro* transcription/translation and cloned into a pCDNA3.1 expression vector (Invitrogen, San Diego, California). For production of recombinant GST-PTIP BRCT domains and GSTBRCA1 BRCT domains, residues 550-757 of PTIP and residues 1634-1863 of BRCA1 were ligated into the EcoRI and Not1 sites of pGEX-4T1 (Pharmacia, Peapack, NJ) and subsequently transformed into DH5a *E. Coli.* Protein induction occurred at 37°C for 4 hours or at 25°C for 16 hours in the presence of 0.4 mM IPTG. For peptide filter blot analysis and measurements of peptide binding affinity by ITC, GSTPTIP BRCT domains (residues 550-757) and GST-BRCA1 BRCT domains (residues 1634-1863) were isolated from bacterial lysates using glutathione agarose, eluted with 40mM glutathione, and dialyzed into 50mM Tris/HCl (pH 8.1), 300mM NaCl. The GFP-PTIP constructs FL (residues 1-757), !BRCT (residues 1-550), or

(BRCT)2 (residues 550-757) were cloned into the EcoRI and Sal1 sites of the pEGFP-C2 (BD Biosciences Clontech Franklin Lakes, NJ) expression vector.

Peptide library screening

Phosphoserine and phosphothreonine oriented degenerate peptide libraries consisting of the sequences Gly-Ala-X-X-X-B-(pSer/pThr)-Gln-J-X-X-X-Ala-Lys-Lys-Lys SEQ ID NO:~~59~~ 62, Met-Ala-X-X-X-X-pThr-X-X-X-X-Ala-Lys-Lys-Lys SEQ ID NO:~~60~~ 47, and Met-Ala-X-X-X-XpSer-X-X-X-X-X-Ala-Lys-Lys-Lys SEQ ID NO:~~61~~ 58; where pS is phosphoserine, pT is phosphothreonine; and X denotes all amino acids except Cys. In the (pSer/pThr)-Gln library, B is a biased mixture of the amino acids A, I, L, M, N, P, S, T, V, and J represents a biased mixture of 25% E, 75% X, where X denotes all amino acids except Arg, Cys, His, Lys. Peptides were synthesized using N-α-FMOC-protected amino acids and standard BOP/HOBt coupling chemistry. Peptide library screening was performed using 125 µl of glutathione beads containing saturating amounts of GST-PTIP BRCT or GST-BRCA1 BRCT domains (1-1.5 mg) as described by Yaffe and Cantley (*Methods Enzymol* 328:157-70, 2000). Beads were packed in a 1mL column and incubated with 0.45 mg of the peptide library mixture for 10 minutes at room temperature in PBS (150 mM NaCl, 3 mM KCl, 10 mM Na2HPO4, 2 mm KH2PO4, pH 7.6). Unbound peptides were removed from the column by two washes with PBS containing 1.0% NP-40 followed by two washes with PBS. Bound peptides were eluted with 30% acetic acid for 10 minutes at room temperature, lyophilized, resuspended in H2O, and sequenced by automated Edman degradation on a PROCISE protein microsequencer (Perkin-Elmer Corporation, Norwalk CT). Selectivity values for each amino acid were determined by comparing the relative abundance (mole percentage) of each amino acid at a particular sequencing cycle in the recovered peptides to that of each amino acid in the original peptide library mixture at the same position.

Isothermal Titration Calorimetry

Peptides were synthesized by solid phase technique with three C-terminal lysines to enhance solubility. The peptides were then purified by reverse phase HPLC following deprotection and confirmed by MALDI-TOF mass spectrometry. Calorimetry measurements were performed using a VP-ITC microcalorimeter (MicroCal Inc., Studio City, CA). Experiments involved serial 10μL injections of peptide solutions (20μM-150μM) into a sample cell containing 15μM GST-PTIP BRCT domains (residues 550-757) or 15μM GST-BRCA1 BRCT domains (residues 1634-1863) in 50mM Tris/HCl (pH 8.1), 300mM NaCl. Twenty injections were performed with 240 second intervals between injections and a reference power of 25 μCal/s. Binding isotherms were plotted and analyzed using ORIGIN Software (MicroCal Inc. Studio City, CA).

Peptide Filter Array

An ABIMED peptide arrayer with a computer controlled Gilson diluter and liquid handling robot (Abimed GmbH, Dusseldorf, Germany) was used to synthesize peptides onto an amino-PEG cellulose membrane using N-a-FMOC-protected amino acids and DIC/HOBT coupling chemistry. The membranes were blocked in 5% milk/TBS-T (0.1%) for 1hour at room temperature, incubated with 0.05 μM GST-PTIP BRCT domains (residues 550-757) or GST-BRCA1 BRCT domains (residues 1634-1863) in 5% milk, 50 mM Tris/HCl (pH 7.6), 150 mM NaCl, 2 mM EDTA, 2mM DTT for 1 hour at room temperature and washed four times with TBS-T (0.1%). The membranes were then incubated with anti-GST conjugated HRP (Amersham) in 5% milk/TBS-T (0.1%) for 1 hour at room temperature, washed five times with TBS-T (0.1%), and subjected to chemiluminescence.

PTIP BRCT domains and BRCA1 BRCT domains binding to cellular substrates

U2OS cells were either treated with 10 Gy of ionizing radiation or mock irradiated and allowed to recover for 30-120 minutes. Cells were subsequently lysed in 50 mM Tris/HCl (pH7.6), 150 mM NaCl, 1.0% NP-40, 5 mM EDTA, 2 mM DTT, 8 μg/mL pepstatin, 8 μg/mL aprotinin, 8 μg/mL leupeptin, 2 mM Na3VO4, 10 mM NaF, 1 μM microcystin. The lysates (0.5-2mg) were incubated with 20 μL glutathione beads containing 10-20 μg of GST-PTIP BRCT domains (residues 550-757), GST-BRCA1 BRCT domains (residues 1634-1863), or GST for 120 minutes at 4°C. Beads were washed three times with lysis buffer. Precipitated proteins were eluted in sample buffer and detected by blotting with anti-ATM/ATR substrate (pSer/pThr)Gln antibody (CELL SIGNALING TECHNOLOGY, Inc Beverly, MA), polyclonal anti-53BP1 (ONCOGENE RESEARCH PRODUCTS, San Diego, California 92121), or monoclonal anti-HA (COVANCE Inc, Princeton, NJ). For peptide competition experiments, GST-PTIP BRCT domains or GST-BRCA1 BRCT domains were immobilized on glutathionine beads and preincubated with 350 μM of BRCTtide-optimal, 7pT, 7T, pSQ-library, SQ-library, or pTP-library for 1 hour at 4°C and washed three times with lysis buffer.

Immunofluorescence and Microscopy

U2OS cells were seeded onto 18mm2 coverslips and transfected with GFP-PTIP constructs FL (residues 1-757), !BRCT (residues 1-550), or (BRCT)2 (residues 550-757) using FUGENE6 transfection reagent (Roche, Basel, Switzerland) according to manufacture's protocol. Twenty-four hours following transfection, the cells were either treated with 10 Gy of ionizing radiation or mock irradiated and allowed to recover for 120 minutes. Cells were fixed in 3% paraformaldehyde/2% sucrose for 15 minutes at room temperature and extracted with a 0.5% Triton X-100 solution containing 20 mM Tris-HCl (pH 7.8), 75 mM

NaCl, 300 mM sucrose, and 3 mM MgCl2 for 15 minutes at room temperature. Slides were stained with primary antibodies at 4°C overnight, then stained with a Texas Red conjugated anti-mouse or anti-rabbit secondary antibody for 60 minutes (Molecular Probes, Eugene, OR) at room temperature. Primary antibodies used were rabbit anti-53BP1 (Oncogene Research Products, San Diego, California), mouse anti-g-H2AX (Upstate, Charlottesville, VA), and rabbit anti-(pS/pT)Q (Cell Signaling Technology, Inc., Beverly, MA). Images were collected on a Deltavision microscope (Carl Zeiss, Thornwood, NY) and digitally deconvolved using SOFTWORX graphics processing software (SGI, CSIF, Stanford, CA).

Peptidomimetics

Peptide derivatives (e.g. peptidomimetics) include cyclic peptides, peptides obtained by substitution of a natural amino acid residue by the corresponding D-stereoisomer, or by a unnatural amino acid residue, chemical derivatives of the peptides, dual peptides, multimers of the peptides, and peptides fused to other proteins or carriers. A cyclic derivative of a peptide of the invention is one having two or more additional amino acid residues suitable for cyclization. These residues are often added at the carboxyl terminus and at the amino terminus. A peptide derivative may have one or more amino acid residues replaced by the corresponding D-amino acid residue. In one example, a peptide or peptide derivative of the invention is all-L, all-D, or a mixed D,L-peptide. In another example, an amino acid residue is replaced by a unnatural amino acid residue. Examples of unnatural or derivatized unnatural amino acids include Nα-methyl amino acids, Cα -methyl amino acids, and β-methyl amino acids.

A chemical derivative of a peptide of the invention includes, but is not limited to, a derivative containing additional chemical moieties not normally a part of the peptide. Examples of such derivatives include: (a) N-acyl derivatives of the amino terminal or of another free amino group, where the acyl group may be either an alkanoyl group, e.g., acetyl, hexanoyl, octanoyl, an aroyl group, e.g.,

benzoyl, or a blocking group such as Fmoc (fluorenylmethyl-O--CO--), carbobenzoxy (benzyl-O--CO--), monomethoxysuccinyl, naphthyl-NH--CO--, acetylamino-caproyl, adamantyl-NH--CO--; (b) esters of the carboxyl terminal or of another free carboxyl or hydroxy groups; (c) amides of the carboxyl terminal or of another free carboxyl groups produced by reaction with ammonia or with a suitable amine; (d) glycosylated derivatives; (e) phosphorylated derivatives; (f) derivatives conjugated to lipophilic moieties, e.g., caproyl, lauryl, stearoyl; and (g) derivatives conjugated to an antibody or other biological ligand. Also included among the chemical derivatives are those derivatives obtained by modification of the peptide bond --CO--NH--, for example, by: (a) reduction to --CH_2 --NH--; (b) alkylation to --CO--N(alkyl)--; and (c) inversion to --NH--CO--.

A dual peptide of the invention consists of two of the same, or two different, peptides of the invention covalently linked to one another, either directly or through a spacer.

Multimers of the invention consist of polymer molecules formed from a number of the same or different peptides or derivatives thereof.

In one example, a peptide derivative is more resistant to proteolytic degradation than the corresponding non-derivatized peptide. For example, a peptide derivative having D-amino acid substitution(s) in place of one or more L-amino acid residue(s) resists proteolytic cleavage.

In another example, the peptide derivative has increased permeability across a cell membrane as compared to the corresponding non-derivatized peptide. For example, a peptide derivative may have a lipophilic moiety coupled at the amino terminus and/or carboxyl terminus and/or an internal site. Such derivatives are highly preferred when targeting intracellular protein-protein interactions, provided they retain the desired functional activity.

In another example, a peptide derivative binds with increased affinity to a ligand (e.g., a Polo box domain).

The peptides or peptide derivatives of the invention are obtained by any method of peptide synthesis known to those skilled in the art, including synthetic and recombinant techniques. For example, the peptides or peptide derivatives can be obtained by solid phase peptide synthesis which, in brief, consists of coupling the carboxyl group of the C-terminal amino acid to a resin and successively adding N-alpha protected amino acids. The protecting groups may be any such groups known in the art. Before each new amino acid is added to the growing chain, the protecting group of the previous amino acid added to the chain is removed. The coupling of amino acids to appropriate resins has been described by Rivier et al. (*U.S. Pat. No.* 4,244,946). Such solid phase syntheses have been described, for example, by Merrifield, *J. Am. Chem. Soc.* 85:2149, 1964; Vale et al., *Science* 213:1394-1397, 1984; Marki et al., *J. Am. Chem. Soc.* 10:3178, 1981, and in *U. S. Pat. Nos.* 4,305,872 and 4,316,891. In a preferred aspect, an automated peptide synthesizer is employed.

Purification of the synthesized peptides or peptide derivatives is carried out by standard methods, including chromatography (e.g., ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, hydrophobicity, or by any other standard technique for the purification of proteins. In one embodiment, thin layer chromatography is employed. In another embodiment, reverse phase HPLC (high performance liquid chromatography) is employed.

Finally, structure-function relationships determined from the peptides, peptide derivatives, and other small molecules of the invention may also be used to prepare analogous molecular structures having similar properties. Thus, the invention is contemplated to include molecules in addition to those expressly disclosed that share the structure, hydrophobicity, charge characteristics and side chain properties of the specific embodiments exemplified herein.

In one example, such derivatives or analogs that have the desired binding activity can be used for binding to a molecule or other target of interest, such as

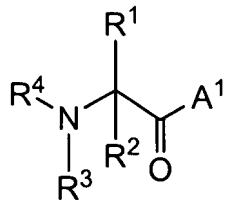
any Polo-box domain. Derivatives or analogs that retain, or alternatively lack or inhibit, a desired property-of-interest (e.g., inhibit PBD binding to a natural ligand), can be used to inhibit the biological activity of a Polo-like kinase (e.g., Plk-1, 2, or 3).

In particular, peptide derivatives are made by altering amino acid sequences by substitutions, additions, or deletions that provide for functionally equivalent molecules, or for functionally enhanced or diminished molecules, as desired. Due to the degeneracy of the genetic code, other nucleic acid sequences that encode substantially the same amino acid sequence may be used for the production of recombinant peptides. These include, but are not limited to, nucleotide sequences comprising all or portions of a peptide of the invention that is altered by the substitution of different codons that encode a functionally equivalent amino acid residue within the sequence, thus producing a silent change.

The derivatives and analogs of the invention can be produced by various methods known in the art. The manipulations that result in their production can occur at the gene or protein level. For example, a cloned nucleic acid sequence can be modified by any of numerous strategies known in the art (Sambrook et al., 1989, Molecular Cloning, *A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). The sequence can be cleaved at appropriate sites with restriction endonuclease(s), followed by further enzymatic modification if desired, isolated, and ligated *in vitro*.

Modified Phosphopeptides

A phosphopeptide of the invention may include, but it is not limited to, an unnatural N-terminal amino acid of the formula (III):

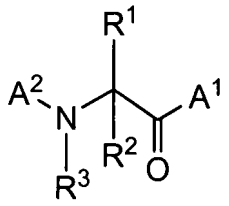


(III) ,

where A^1 is an amino acid or peptide chain linked via an α-amino group; R^1 and R^3 are independently hydrogen, C_{1-5} branched or linear C_{1-5} alkyl, C_{1-5} alkaryl, heteroaryl, and aryl, each of which are unsubstituted or substituted with a substitutent selected from: 1 to 3 of C_{1-5} alkyl, 1 to 3 of halogen, 1 to 2 of $-OR^5$, $N(R^5)(R^6)$, SR^5, $N\text{-}C(NR^5)NR^6R^7$, methylenedioxy, $-S(O)_mR^5$, 1 to 2 of $-CF_3$, $-OCF_3$, nitro, $-N(R^5)C(O)(R^6)$, $-C(O)OR^5$, $-C(O)N(R^5)(R^6)$, -1H-tetrazol-5-yl, $-SO_2N(R^5)(R^6)$, $-N(R^5)SO_2$ aryl, or $-N(R^5)SO_2R^6$; R^5, R^6 and R^7 are independently selected from hydrogen, C_{1-5} linear or branched alkyl, C_{1-5} alkaryl, aryl, heteroaryl, and C_{3-7} cycloalkyl, and where two C_{1-5} alkyl groups are present on one atom, they optionally are joined to form a C_{3-8} cyclic ring, optionally including oxygen, sulfur or NR^7, where R^7 is hydrogen, or C_{1-5} alkyl, optionally substituted by hydroxyl; R^2 is hydrogen, F, C_{1-5} linear or branched alkyl, C_{1-5} alkaryl; or R^2 and R^1 are joined to form a C_{3-8} cyclic ring, optionally including oxygen, sulfur, or NR^7, where R^7 is hydrogen, or C_{1-5} alkyl, optionally substituted by hydroxyl, or R^2 and R^3 are joined to form a C_{3-8} cyclic ring, optionally substituted by hydroxyl and optionally including oxygen, sulfur or NR^7, where R^7 is hydrogen, or C_{1-5} alkyl; R^2 is hydrogen, F, C_{1-5} linear or branched alkyl, C_{1-5} alkaryl; and R^4 is hydrogen, C_{1-5} branched or linear C_{1-5} alkyl, C_{1-5} alkaryl, heteroaryl, and aryl, each of which are unsubstituted or substituted with a substitutent selected from: 1 to 3 of C_{1-5} alkyl, 1 to 3 of halogen, 1 to 2 of $-OR^5$, $N(R^5)(R^6)$, $N\text{-}C(NR^5)NR^6R^7$, methylenedioxy, $-S(O)_mR^5$ (where m is 0-2), 1 to 2 of $-CF_3$, $-OCF_3$, nitro, $-N(R^5)C(O)(R^6)$, $-N(R^5)C(O)(OR^6)$, $-C(O)OR^5$, $-C(O)N(R^5)(R^6)$, -1H-tetrazol-5-yl, $-SO_2N(R^5)(R^6)$, $-N(R^5)SO_2$ aryl, or $-N(R^5)SO_2R^6$, R^5, R^6 and R^7 are independently selected from hydrogen, C_{1-5} linear or branched alkyl, C_{1-5} alkaryl, aryl, heteroaryl, and C_{3-7}

cycloalkyl, and where two C_{1-5} alkyl groups are present on one atom, they optionally are joined to form a C_{3-8} cyclic ring, optionally including oxygen, sulfur or NR^7, where R^7 is hydrogen, or C_{1-5} alkyl, optionally substituted by hydroxyl.

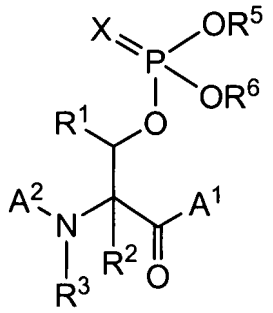
The phosphopeptides of the invention may also include an internal unnatural internal amino acid of the formula:



(IV) ,

where A^2 is an amino acid or peptide chain linked via an α-carboxy group; A^1 is an amino acid or peptide chain linked via an α-amino group; R^1 and R^3 are independently hydrogen, C_{1-5} branched or linear C_{1-5} alkyl, C_{1-5} alkaryl, heteroaryl, and aryl, each of which are unsubstituted or substituted with a substitutent selected from: 1 to 3 of C_{1-5} alkyl, 1 to 3 of halogen, 1 to 2 of $-OR^5$, $N(R^5)(R^6)$, SR^5, $-C(NR^5)NR^6R^7$, methylenedioxy, $-S(O)_mR^5$ (m is 1-2), 1 to 2 of $-CF_3$, $-OCF_3$, nitro, $-N(R^5)C(O)(R^6)$, $-C(O)OR^5$, $-C(O)N(R^5)(R^6)$, -1H-tetrazol-5-yl, $-SO_2N(R^5)(R^6)$, $-N(R^5)SO_2$ aryl, or $-N(R^5)SO_2R^6$; R^5, R^6 and R^7 are independently selected from hydrogen, C_{1-5} linear or branched alkyl, C_{1-5} alkaryl, aryl, heteroaryl, and C_{3-7} cycloalkyl, and where two C_{1-5} alkyl groups are present on one atom, they optionally are joined to form a C_{3-8} cyclic ring, optionally including oxygen, sulfur or NR^7, where R^7 is hydrogen, or C_{1-5} alkyl, optionally substituted by hydroxyl; and R^2 is hydrogen, F, C_{1-5} linear or branched alkyl, C_{1-5} alkaryl; or R^2 and R^1 are joined to form a C_{3-8} cyclic ring, optionally including oxygen, sulfur or NR^7, where R^7 is hydrogen, or C_{1-5} alkyl, optionally substituted by hydroxyl, or R^2 and R^3 are joined to form a C_{3-8} cyclic ring, optionally substituted by hydroxyl and optionally including oxygen, sulfur or NR^7, where R^7 is hydrogen, or C_{1-5} alkyl.

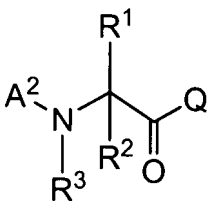
The invention also includes modifications of the phosphopeptides of the invention, wherein an internal unnatural internal amino acid of the formula:



(V)

is present, where A^2 is an amino acid or peptide chain linked via an α-carboxy group; A^1 is an amino acid or peptide chain linked via an α-amino group; R^1 and R^3 are independently hydrogen, C_{1-5} branched or linear C_{1-5} alkyl, and C_{1-5} alkaryl; R^2 is hydrogen, F, C_{1-5} linear or branched alkyl, C_{1-5} alkaryl; or R^2 and R^1 are joined to form a C_{3-8} cyclic ring, optionally including oxygen, sulfur or NR^7, where R^7 is hydrogen, or C_{1-5} alkyl, optionally substituted by hydroxyl; X is O or S; and R^5 and R^6 are independently selected from hydrogen, C_{1-5} linear or branched alkyl, C_{1-5} alkaryl, aryl, heteroaryl, and C_{3-7} cycloalkyl, and where two C_{1-5} alkyl groups are present on one atom, they optionally are joined to form a C_{3-8} cyclic ring, optionally including oxygen, sulfur or NR^7, where R^7 is hydrogen, or C_{1-5} alkyl, optionally substituted by hydroxyl; or R^5 and R^6 are joined to form a C_{3-8} cyclic ring, optionally including oxygen, sulfur or NR^7, where R^7 is hydrogen, or C_{1-5} alkyl, optionally substituted by hydroxyl.

The phosphopeptides of the invention may also include a C-terminal unnatural internal amino acid of the formula:



(VI) ,

where A^2 is an amino acid or peptide chain linked via an α-carboxy group; R^1 and R^3 are independently hydrogen, C_{1-5} branched or linear C_{1-5} alkyl, C_{1-5} alkaryl, heteroaryl, and aryl, each of which are unsubstituted or substituted with a substitutent selected from: 1 to 3 of C_{1-5} alkyl, 1 to 3 of halogen, 1 to 2 of $-OR^5$, $N(R^5)(R^6)$, SR^5, $N\text{-}C(NR^5)NR^6R^7$, methylenedioxy, $-S(O)_mR^5$, 1 to 2 of $-CF_3$, $-OCF_3$, nitro, $-N(R^5)C(O)(R^6)$, $-C(O)OR^5$, $-C(O)N(R^5)(R^6)$, -1H-tetrazol-5-yl, $-SO_2N(R^5)(R^6)$, $-N(R^5)SO_2$ aryl, or $-N(R^5)SO_2R^6$; R^5, R^6 and R^7 are independently selected from hydrogen, C_{1-5} linear or branched alkyl, C_{1-5} alkaryl, aryl, heteroaryl, and C_{3-7} cycloalkyl, and where two C_{1-5} alkyl groups are present on one atom, they optionally are joined to form a C_{3-8} cyclic ring, optionally including oxygen, sulfur or NR^7, where R^7 is hydrogen, or C_{1-5} alkyl, optionally substituted by hydroxyl; R^2 is hydrogen, F, C_{1-5} linear or branched alkyl, C_{1-5} alkaryl; or R^2 and R^1 are joined to form a C_{3-8} cyclic ring, optionally including oxygen, sulfur or NR^7, where R^7 is hydrogen, or C_{1-5} alkyl, optionally substituted by hydroxyl; or R^2 and R^3 are joined to form a C_{3-8} cyclic ring, optionally substituted by hydroxyl and optionally including oxygen, sulfur or NR^7, where R^7 is hydrogen, or C_{1-5} alkyl; R^2 is hydrogen, F, C_{1-5} linear or branched alkyl, C_{1-5} alkaryl; and Q is OH , OR^5, or NR^5R^6, where R^5, R^6 are independently selected from hydrogen, C_{1-5} linear or branched alkyl, C_{1-5} alkaryl, aryl, heteroaryl, and C_{3-7} cycloalkyl, and where two C_{1-5} alkyl groups are present on one atom, they optionally are joined to form a C_{3-8} cyclic ring, optionally including oxygen, sulfur or NR^7, where R^7 is hydrogen, or C_{1-5} alkyl, optionally substituted by hydroxyl. Methods well known in the art for modifying peptides are found, for example, in "Remington: The Science and Practice of Pharmacy" (20th ed., ed. A.R. Gennaro, 2000, Lippincott Williams & Wilkins, Philadelphia).

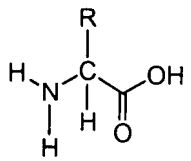
Therapeutic Uses

Peptide synthesis and conjugation

Phosphopeptides of the invention are prepared as detailed above. Alternatively, phosphopeptides can be prepared using standard FMOC chemistry on 2-chlorotrityl chloride resin (Int. J. Pept. Prot. Res. 38, 1991, 555-61). Cleavage from the resin is performed using 20% acetic acid in dichloromehane (DCM), which leaves the side chain still blocked. Free terminal carboxylate peptide is then coupled to 4'(aminomethy)-fluorescein (Molecular Probes, A-1351; Eugene, OR) using excess diisopropylcarbodiimide (DIC) in dimethylformamide (DMF) at room temperature. The fluorescent N-C blocked peptide is purified by silica gel chromatography (10% methanol in DCM). The N terminal FMOC group is then removed using piperidine (20%) in DMF, and the N-free peptide, purified by silica gel chromatography (20% methanol in DCM, 0.5% HOAc). Finally, any t-butyl side chain protective groups are removed using 95% trifluoroacetic acid containing 2.5 % water and 2.5 % triisopropyl silane. The peptide obtained in such a manner should give a single peak by HPLC and is sufficiently pure for carrying on with the assay described below.

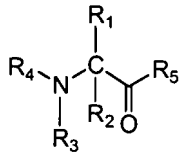
Phosphopeptide Modifications

It is understood that modifications can be made to the amino acid residues of the phosphopeptides of the invention, to enhance or prolong the therapeutic efficacy and/or bioavailability of the phosphopeptide. Accordingly, α-amino acids having the following general formula (I):



(I)

where R defines the specific amino acid residue, may undergo various modifications. Exemplary modifications of α-amino acids, include, but are not limited to, the following formula (II):



(II)

R_1, R_2, R_3, R_4, and R_5, are independently hydrogen, hydroxy, nitro, halo, C_{1-5} branched or linear alkyl, C_{1-5} alkaryl, heteroaryl, and aryl; wherein the alkyl, alkaryl, heteroaryl, and aryl may be unsubstituted or substituted by one or more substituents selected from the group consisting of C_{1-5} alkyl, hydroxy, halo, nitro, C_{1-5} alkoxy, C_{1-5} alkylthio, trihalomethyl, C_{1-5} acyl, arylcarbonyl, heteroarylcarbonyl, nitrile, C_{1-5} alkoxycarbonyl, oxo, arylalkyl (wherein the alkyl group has from 1 to 5 carbon atoms) and heteroarylalkyl (wherein the alkyl group has from 1 to 5carbon atoms); alternatively, R_1 and R_2 are joined to form a C_{3-8} cyclic ring, optionally including oxygen, sulfur or hydrogen, or C_{1-5} alkyl, optionally substituted by hydroxyl; or R_2 and R_3 are joined to form a C_{3-8} cyclic ring, optionally substituted by hydroxyl and optionally including oxygen, sulfur, C_{1-5} aminoalkyl, or C_{1-5} alkyl. Methods well known in the art for making modifications are found, for example, in "Remington: The Science and Practice of Pharmacy" (20th ed., ed. A.R. Gennaro, 2000, Lippincott Williams & Wilkins), hereby incorporated by reference.

Assays and high throughput assays

Fluorescence polarization assays can be used in displacement assays to identify small molecule peptidomimetics. The following is an exemplary method for use of fluorescence polarization, and should not be viewed as limiting in any way. For screening, all reagents are diluted at the appropriate concentration and the working solution, kept on ice. The working stock concentration for GST and

GST fusion proteins are ~4 ng/μL, Fluorescein-labeled phosphopeptides can be used at a concentration of 1.56 fmol/μL, while cold phosphopeptides and peptides at 25 pmol/μL. Samples are incubated at a total volume of 200 μL per well in black flat bottom plates, Biocoat, #359135 low binding (BD BioSciences; Bedford, MA). Assays are started with the successive addition using a Labsystem Multi-Drop 96/384 device (Labsystem; Franklin, MA) of 50μL test compounds, diluted in 10% DMSO (average concentration of 28 μM), 50μL of 50 mM MES-pH 6.5, 50μL of Fluorescein-phosphopeptide, 50μL of GST-Plk-1 PBD, 50μL of unlabeled phosphopeptide, or unphosphorylated peptide can be used as a negative control. Once added, all the plates are placed at 4°C. Following overnight incubation at 4°C, the fluorescence polarization is measured using a Polarion plate reader (Tecan, Research Triangle Park, NC). A xenon flash lamp equipped with an excitation filter of 485 nm and an emission filter of 535 nm. The number of flashes is set at 30. Raw data can then be converted into a percentage of total interaction(s). All further analysis can be performed using SPOTFIRE data analysis software (SPOTFIRE, Somerville, MA)

Upon selection of active compounds, auto-fluorescence of the hits is measured as well as the fluorescein quenching effect, where a measurement of 2000 or more units indicates auto-fluorescence, while a measurement of 50 units indicates a quenching effect. Confirmed hits can then be analyzed in dose-response curves (IC_{50}) for reconfirmation. Best hits in dose–response curves can then be assessed by isothermal titration calorimetry using GST-Plk-1 PBD.

Alternate binding and displacement assays

Fluorescence polarization assays are but one means to measure phosphopeptide-protein interactions in a screening strategy. Alternate methods for measuring phosphopeptide-protein interactions are known to the skilled artisan. Such methods include, but are not limited to mass spectrometry (Nelson and Krone, J. Mol. Recognit., 12:77-93, 1999), surface plasmon resonance (Spiga *et*

al., FEBS Lett., 511:33-35, 2002; Rich and Mizka, J. Mol. Recognit., 14:223-8, 2001; Abrantes *et al.*, Anal. Chem., 73:2828-35, 2001), fluorescence resonance energy transfer (FRET) (Bader *et al.*, J. Biomol. Screen, 6:255-64, 2001; Song *et al.*, Anal. Biochem. 291:133-41, 2001; Brockhoff et al., Cytometry, 44:338-48, 2001), bioluminescence resonance energy transfer (BRET) (Angers *et al.*, *Proc. Natl. Acad. Sci. USA*, 97:3684-9, 2000; Xu *et al.*, *Proc. Natl. Acad. Sci. USA*, 96:151-6, 1999), fluorescence quenching (Engelborghs, *Spectrochim. Acta A. Mol. Biomol. Spectrosc.*, 57:2255-70, 70; Geoghegan *et al.*, *Bioconjug. Chem.* 11:71-7, 2000), fluorescence activated cell scanning/sorting (Barth *et al., J. Mol. Biol.*, 301:751-7, 2000), ELISA, and radioimmunoassay (RIA).

Test extracts and compounds

In general, peptidomimetic compounds that affect phosphopeptide-protein interactions are identified from large libraries of both natural products, synthetic (or semi-synthetic) extracts or chemical libraries, according to methods known in the art.

Those skilled in the art will understand that the precise source of test extracts or compounds is not critical to the screening procedure(s) of the invention. Accordingly, virtually any number of chemical extracts or compounds can be screened using the exemplary methods described herein. Examples of such extracts or compounds include, but are not limited to, plant-, fungal-, prokaryotic- or animal-based extracts, fermentation broths, and synthetic compounds, as well as modifications of existing compounds. Numerous methods are also available for generating random or directed synthesis (e.g., semi-synthesis or total synthesis) of any number of chemical compounds, including, but not limited to, saccharide-, lipid-, peptide-, and nucleic acid-based compounds. Synthetic compound libraries are commercially available from, for example, Brandon Associates (Merrimack, NH) and Aldrich Chemical (Milwaukee, WI)

Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant, and animal extracts are commercially available from a number of sources, including, but not limited to, Biotics (Sussex, UK), Xenova (Slough, UK), Harbor Branch Oceangraphics Institute (Ft. Pierce, FL), and PharmaMar, U.S.A. (Cambridge, MA). In addition, natural and synthetically produced libraries are produced, if desired, according to methods known in the art (e.g., by combinatorial chemistry methods or standard extraction and fractionation methods). Furthermore, if desired, any library or compound may be readily modified using standard chemical, physical, or biochemical methods.

Administration of phosphopeptides and peptidomimetic small molecules

By selectively disrupting or preventing a phosphoprotein from binding to its natural partner(s) through its binding site, the phosphopeptides of the invention, or derivatives, or peptidomimetics thereof, can significantly alter the biological activity or the biological function of a polo-like kinase. Therefore, the phosphopeptides, or derivatives thereof, of the invention can be used for the treatment of a disease or disorder characterized by inappropriate cell cycle regulation or apoptosis.

Diseases or disorders characterized by inappropriate cell cycle regulation, include hyperproliferative disorders, such as neoplasias. Examples of neoplasms include, without limitation, leukemias (e.g., acute leukemia, acute lymphocytic leukemia, acute myelocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroleukemia, chronic leukemia, chronic myelocytic leukemia, chronic lymphocytic leukemia), polycythemia vera, lymphoma (Hodgkin's disease, non-Hodgkin's disease), Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors such as sarcomas and carcinomas (e.g., fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma,

lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, uterine cancer, testicular cancer, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodenriglioma, schwannoma, meningioma, melanoma, neuroblastoma, and retinoblastoma).

Cells undergoing inappropriate apoptosis include neurons in a patient who has a neurodegenerative disease (e.g., Parkinson's disease, Alzheimer's disease, or stroke), and cardiomyocytes (e.g., after myocardial infarction or over the course of congestive heart failure). Compositions of the invention, i.e., inhibitors of Plk-3, may be useful in treating a cell undergoing inappropriate apoptosis.

A Plk-1 PBD-binding phosphopeptide or peptidomimetic small molecule may be administered within a pharmaceutically-acceptable diluent, carrier, or excipient, in unit dosage form. Conventional pharmaceutical practice may be employed to provide suitable formulations or compositions to administer the compounds to patients suffering from a disease that is caused by excessive cell proliferation. Administration may begin before the patient is symptomatic. Any appropriate route of administration may be employed, for example, administration may be parenteral, intravenous, intra-arterial, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intracapsular, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol, suppository, or oral administration. For example, therapeutic formulations may be in the form of liquid solutions or suspensions; for oral administration, formulations may be in the

form of tablets or capsules; and for intranasal formulations, in the form of powders, nasal drops, or aerosols.

The pharmaceutical compositions of the present invention are prepared in a manner known per se, for example by means of conventional dissolving, lyophilising, mixing, granulating or confectioning processes. Methods well known in the art for making formulations are found, for example, in "Remington: The Science and Practice of Pharmacy" (20th ed., ed. A.R. Gennaro, 2000, Lippincott Williams & Wilkins, Philadelphia).

Solutions of the active ingredient, and also suspensions, and especially isotonic aqueous solutions or suspensions, are preferably used, it being possible, for example in the case of lyophilized compositions that comprise the active ingredient alone or together with a carrier, for example mannitol, for such solutions or suspensions to be produced prior to use. The pharmaceutical compositions may be sterilized and/or may comprise excipients, for example preservatives, stabilisers, wetting and/or emulsifying agents, solubilisers, salts for regulating the osmotic pressure and/or buffers, and are prepared in a manner known per se, for example by means of conventional dissolving or lyophilising processes. The said solutions or suspensions may comprise viscosity-increasing substances, such as sodium carboxymethylcellulose, carboxymethylcellulose, dextran, poly vinylpyrrolidone or gelatin.

Suspensions in oil comprise as the oil component the vegetable, synthetic or semi-synthetic oils customary for injection purposes. There may be mentioned as such especially liquid fatty acid esters that contain as the acid component a long-chained fatty acid having from 8 to 22, especially from 12 to 22, carbon atoms, for example lauric acid, tridecylic acid, myristic acid, pentadecylic acid, palmitic acid, margaric acid, stearic acid, arachidic acid, behenic acid or corresponding unsaturated acids, for example oleic acid, elaidic acid, erucic acid, brasidic acid or linoleic acid, if desired with the addition of anti oxidants, for example, vitamins E, β-carotene, or 3,5-di-tert-butyl-4-hydroxytoluene. The

alcohol component of those fatty acid esters has a maximum of 6 carbon atoms and is a mono- or poly-hydroxy, for example a mono-, di- or tri-hydroxy, alcohol, for example methanol, ethanol, propanol, butanol or pentanol or the isomers thereof, but especially glycol and glycerol. The following examples of fatty acid esters are there fore to be mentioned: ethyl oleate, isopropyl myristate, isopropyl palmitate, "Labrafil M 2375" (poly oxyethylene glycerol trioleate, Gattefoss, Paris), "Miglyol 812" (triglyceride of saturated fatty acids with a chain length of C_8 to C_{12}, Huls AG, Germany), but especially vegetable oils, such as cottonseed oil, almond oil, olive oil, castor oil, sesame oil, soybean oil and more especially groundnut oil.

The injection compositions are prepared in customary manner under sterile conditions; the same applies also to introducing the compositions into ampoules or vials and sealing the containers.

Pharmaceutical compositions for oral administration can be obtained by combining the active ingredient with solid carriers, if desired granulating a resulting mixture, and processing the mixture, if desired or necessary, after the addition of appropriate excipients, into tablets, drage cores or capsules. It is also possible for them to be incorporated into plastics carriers that allow the active ingredients to diffuse or be released in measured amounts.

Suitable carriers are especially fillers, such as sugars, for example lactose, saccharose, mannitol or sorbitol, cellulose preparations and/or calcium phosphates, for example tricalcium phosphate or calcium hydrogen phosphate, and binders, such as starch pastes using for example corn, wheat, rice or potato starch, gelatin, tragacanth, methylcellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose and/or polyvinyl-pyrrolidone, and/or, if desired, disintegrates, such as the above-mentioned starches, also carboxymethyl starch, crosslinked polyvinylpyrrolidone, agar, alginic acid or a salt thereof, such as sodium alginate. Excipients are especially flow conditioners and lubricants, for example silicic acid, talc, stearic acid or salts thereof, such as magnesium or

calcium stearate, and/or polyethylene glycol. Drage cores are provided with suitable, optionally enteric, coatings, there being used, *inter alia*, concentrated sugar solutions which may comprise gum arabic, talc, polyvinylpyrrolidone, polyethylene glycol and/or titanium dioxide, or coating solutions in suitable organic solvents, or, for the preparation of enteric coatings, solutions of suitable cellulose preparations, such as ethylcellulose phthalate or hydroxypropylmethylcellulose phthalate. Capsules are dry-filled capsules made of gelatin and soft sealed capsules made of gelatin and a plasticiser, such as glycerol or sorbitol. The dry-filled capsules may comprise the active ingredient in the form of granules, for example with fillers, such as lactose, binders, such as starches, and/or glidants, such as talc or magnesium stearate, and if desired with stabilisers. In soft capsules the active ingredient is preferably dissolved or suspended in suitable oily excipients, such as fatty oils, paraffin oil or liquid polyethylene glycols, it being possible also for stabilisers and/or antibacterial agents to be added. Dyes or pigments may be added to the tablets or drage coatings or the capsule casings, for example for identification purposes or to indicate different doses of active ingredient.

The pharmaceutical compositions comprise from approximately 1% to approximately 95%, preferably from approximately 20% to approximately 90%, active ingredient. Pharmaceutical compositions according to the invention may be, for example, in unit dose form, such as in the form of ampoules, vials, suppositories, drages, tablets or capsules.

The formulations can be administered to human patients in a therapeutically effective amount (e.g., an amount that decreases, suppresses, attenuates, diminishes, arrests, or stabilizes the development or progression of a disease, disorder, or infection in a eukaryotic host organism). The preferred dosage of therapeutic agent to be administered is likely to depend on such variables as the type and extent of the disorder, the overall health status of the particular patient, the formulation of the compound excipients, and its route of administration.

For any of the methods of application described above, a Plk-1 PBD-interacting small molecule may be applied to the site of the needed therapeutic event (for example, by injection), or to tissue in the vicinity of the predicted therapeutic event or to a blood vessel supplying the cells predicted to require enhanced therapy.

The dosages of Plk-1 PBD-interacting small molecule(s) depends on a number of factors, including the size and health of the individual patient, but, generally, between 0.1 mg and 1000 mg inclusive are administered per day to an adult in any pharmaceutically acceptable formulation. In addition, treatment by any of the approaches described herein may be combined with more traditional therapies.

Combination Therapy

If desired, treatment with Plk-1 PBD-interacting small molecule may be combined with more traditional therapies for the proliferative disease such as surgery or administration of chemotherapeutics or other anti-cancer agents, including, for example, γ-radiation, alkylating agents (e.g., nitrogen mustards such as cyclophosphamide, ifosfamide, trofosfamide, and chlorambucil; nitrosoureas such as carmustine, and lomustine; alkylsulphonates such as bisulfan and treosulfan; triazenes such as dacarbazine; platinum-containing compounds such as cisplatin and carboplatin), plant alkaloids (e.g., vincristine, vinblastine, anhydrovinblastine, vindesine, vinorelbine, paclitaxel, and docetaxol), DNA topoisomerase inhibitors (e.g., etoposide, teniposide, topotecan, 9-aminocamptothecin, (campto) irinotecan, and crisnatol), mytomycins (e.g., mytomicin C), antifolates (e.g., methotrexate, trimetrexate, mycophenolic acid, tiazofurin, ribavirin, EICAR, hydroxyurea, and deferoxamine), uracil analogs (5-fluorouracil, floxuridine, doxifluridine, and ratitrexed), cytosine analogs (cytarbine, cytosine arabinoside, and fludarabine), purine analogs (e.g.,

mercaptopurine, and thioguanine), hormonal therapies (e.g., tamoxifen, raloxifene, megestrol, goserelin, leuprolide acetate, flutamide, and bicalutamide), vitamin D3 analogs (EB 1089, CB 1093, and KH 1060), vertoporfin, phthalocyanine, photosensitizer Pc4, demethoxy-hypocrellin A, interferon-α, interferon-γ, tumor necrosis factor, lovastatin, 1-methyl-4-phenylpyridinium ion, staurosporine, actinomycin D, dactinomycin, bleomycin A2, bleomycin B2, adriamycin, peplomycin, daunorubican, idarubican, epirubican, pirarubican, zorubican, mitoxantrone, and verapamil.

Other Embodiments

From the foregoing description, it is apparent that variations and modifications may be made to the invention described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

All patents and publications mentioned in this specification are hereby incorporated by reference to the same extent as if each independent publication or patent application, including 60/426,132, was specifically and individually indicated to be incorporated by reference.

What is claimed is: